

Denny J. Meyer

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# Veterinary Laboratory Medicine

*Interpretation  
and  
Diagnosis*

THIRD EDITION

SAUNDERS

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

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Veterinary Laboratory Medicine: Interpretation & Diagnosis

iii

3rd ed.

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# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

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## <sup>2</sup> Chapter 1 Introduction to Laboratory Medicine

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Laboratory medicine (clinical pathology) involves the determination, study, and use of laboratory tests for the evaluation of health and disease. Laboratory medicine depends heavily on basic biochemistry, physiology, and pathology, providing a bridge between the basic sciences and clinical medicine. Disciplines consistently included in the field of laboratory medicine are clinical chemistry, hematology, exfoliative cytology, urinalysis, clinical endocrinology, and clinical immunology. Additional disciplines that may be included as subdivisions of laboratory medicine are clinical toxicology, microbiology, parasitology, and surgical pathology (especially in commercial laboratories). The latter four disciplines are not specifically addressed in this text.

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Laboratory tests are done for a variety of reasons. Screening tests may be done on clinically healthy or normal animals. For example, when an animal is acquired, tests may be performed to assist clients in determining whether they want to make a financial or emotional commitment to a diseased animal. Tests are also performed to examine geriatric patients for subclinical disease and to identify conditions that might make an animal a poor candidate for anesthesia or surgery. Screening tests are often done when an ill animal is first examined, especially if systemic signs of illness are present and a specific diagnosis is not apparent from the history and results of the physical examination. Tests are also done to identify specific organ injury or dysfunction and to confirm a presumptive diagnosis. A test may be repeated, or a different test may be done to confirm a test result that was previously reported to be abnormal. Tests may be done to assist in the determination of the severity of a disease, to help formulate a prognosis, and to monitor the response to therapy or progression of disease.

Decisions to request laboratory tests in animals are largely based on the cost of the test versus the potential benefit of the result to the animal. Certain tests are routinely done to establish a database for patient evaluation, and others are done in an attempt to evaluate a specific problem. Tests regularly done to establish a database include a complete blood count, clinical chemistry profile, and urinalysis. Charges per test are lower when a panel of tests is done than when tests are ordered separately.

Examples of more specific tests that focus on a problem identified during the diagnostic evaluation of an animal include special chemistry tests, such as determination of bile acid levels; coagulation tests, such as prothrombin time; bone marrow biopsy and interpretation; exfoliative cytologic examination of an aspirate smear from a mass; evaluation of a body fluid, such as spinal fluid; immunology tests, such as the direct Coombs' test; microbial culture; serologic tests, such as an *Ehrlichia canis* titer; fecal examination for parasite ova; toxicology tests, such as a blood lead measurement; and surgical biopsy followed by histopathologic examination. Although single tests may be done to address a specific problem (e.g., blood glucose is measured to evaluate response to therapy of a diabetic animal), multiple tests are often done to provide a more comprehensive answer to a broader problem (e.g., a hemostasis panel is generally ordered for evaluation of a bleeding animal).

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*Stat* is an abbreviation for *statim* (Latin meaning “immediately”). Stat tests are tests that are given high priority and begun immediately in situations in which rapid results are needed for the medical management of critically ill patients. Additional fees may be charged for stat tests because they disrupt the flow of work in the laboratory.

### 2.1 INTERNAL VERSUS EXTERNAL LABORATORIES

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A variety of factors should influence the decision about whether a test will be done in an in-house laboratory or be sent to an external laboratory. A major concern is whether the necessary personnel, equipment, and supplies are available to perform the test accurately. Considerations include personnel knowledge of species differences and a willingness to conduct quality control tests to verify that the procedure is working properly. The cost per test

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(technician time, reagent costs, equipment costs) must be compared to determine which option is more economical. The stability of the test may dictate which tests are done in house (e.g., urine sediment structure deteriorates within hours). The time it takes to obtain results may be important, especially for critically ill patients. The hours of operation of the laboratories are important for test results that are needed at night or on the weekend. Commercial laboratories generally have better quality control than laboratories within a private practice.

Commercial veterinary laboratories are preferred to commercial human laboratories because errors can occur if tests designed to evaluate human samples are used without modification for use with animal samples. Some automated analyzers (especially hematology analyzers) must be calibrated for species differences to obtain accurate results. Technologists must be aware that blood cell structure and blood parasites differ by species. Antibody-dependent immunology and endocrinology tests designed for humans may not be valid for animals. Veterinary laboratories are more likely to have established their own reference intervals for various animal species (as opposed to extracting them from the literature) than are human laboratories. Knowledge of specific animal diseases and training in veterinary laboratory medicine are essential for the evaluation of cytologic specimens and interpretation of laboratory data; consequently, a veterinary clinical pathologist should be available to perform certain subjective tests and provide consultation concerning all test results.

## 2.2 REFERENCE INTERVALS

Appropriate reference intervals must be established from apparently healthy animals drawn from the same general population as the ill animals to be examined to interpret laboratory data from ill animals. The term *reference interval* is preferred to the commonly used term *normal range*. The latter term implies that it is the range of test results from all “normal” animals. In reality, a low percentage of apparently healthy “normal” animals will have test values outside the normal range, and, depending on the test, many abnormal (diseased) animals may have values within the normal range. Healthy animals may have transient increases or decreases in laboratory test results because of changes in environment, emotional status, diet, or other factors; and a small percentage of healthy animals simply have values higher than those of the general population of healthy animals. Apparently healthy animals may also have occult disease that causes one or more abnormal laboratory test results; and errors in sample collection, handling, and laboratory procedures can result in artifactual high or low values for healthy animals.

Consequently, it is not appropriate to simply use the actual range of values from all apparently healthy animals evaluated. To develop useful reference intervals, one must decide which animals will be evaluated, how many animals need to be evaluated, and what method(s) will be used to remove high or low outliers that would otherwise render the interval of limited value as a reference.

### 2.2.1 Selection of Reference Animals

Specific reference intervals are needed for each species of animal being tested. Less often, a different reference interval is needed for an analyte from a specific breed of animal (e.g., hematocrit values in greyhounds are higher than those in other dog breeds). Many values vary with the age of the animal, with major changes occurring before puberty (e.g., 3-week-old pups have lower hematocrits and higher serum alkaline phosphatase values than adults). Consequently, some analytes require different reference intervals for different age groups. Some analyte values also vary with sex, pregnancy or egg-laying, diet, geographic location, time of sampling, time after eating, emotional state, and activity level. The type(s) of animals sampled and environmental conditions present during the establishment of a reference interval should be defined along with the methods and equipment used so that the user can make appropriate evaluations. Ideally, a reference interval should be established by using a population of healthy animals with a composition (age, breed, sex, diet, and other factors) like that of the population of ill animals being evaluated. Homogeneous populations generally have more narrow reference intervals than heterogenous populations. Establishing a reference interval for a blood analyte by using a group of

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male foxhounds housed in a research colony, fed the same diet, and conditioned to phlebotomies would likely result in reference intervals too narrow for the population of dogs examined in a typical small animal practice. Reference intervals are generally established for a species by using samples from apparently healthy adult animals of both sexes and various breeds that have been fasted overnight.

### 2.2.2 Determination of Reference Intervals

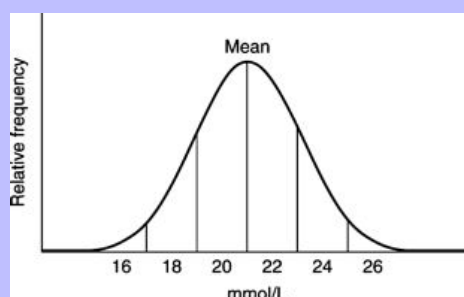
Specific reference intervals should be established for each instrument and each test evaluated. Ideally, each animal would have its own reference intervals established by multiple tests being done over time when it is healthy. In some instances, limited numbers of baseline values are available for an animal, which can be helpful, but rarely are analytes measured often enough to establish an accurate reference interval for an individual animal. Consequently, population-based reference intervals are used.

When the frequency diagram of test results from a healthy population is examined, many analyte values exhibit a Gaussian or bell-shaped distribution (Fig. 1-1). When a Gaussian distribution is present, a minimum of 40 individuals (200 are preferred but rarely available) should be evaluated for statistical validity. In this case, the reference interval is calculated by using the mean  $\pm$  2 SDs. This interval approximates the 95% confidence interval (CI). In other words, about 95% of healthy animals have test values within this reference interval, with about 2.5% of healthy animals having values above and about 2.5% of healthy animals having values below the reference interval. A common mistake made by novices is calculation of the reference interval from the mean  $\pm$  1 SD. When this is done, about 32% of healthy animals will have values outside the calculated interval.

Some analyte values do not exhibit a Gaussian distribution. Most commonly, there is a skew toward the higher values. The use of mean  $\pm$  2 SDs to calculate reference intervals results in inappropriate reference intervals for skewed populations, as shown in Fig. 1-2. Data may be manipulated (e.g., log or square root transformation) so that the frequency distribution of the transformed data approximates a Gaussian distribution. The boundaries are determined as described previously, and results are retransformed to determine the reference interval.

Alternatively, one can use percentiles to determine upper and lower limits, especially if large numbers of healthy animals are evaluated. Values are listed in ascending order. The lower limit is determined by the formula  $(n + 1) \times 0.025$ , and the upper limit is determined by the formula  $(n + 1) \times 0.975$ , where  $n$  = the number of normal animals evaluated. If 119 animals were used, the value for the third animal would be used as the lower limit and the value for the 117th animal (third from the top) would be used as the upper limit.

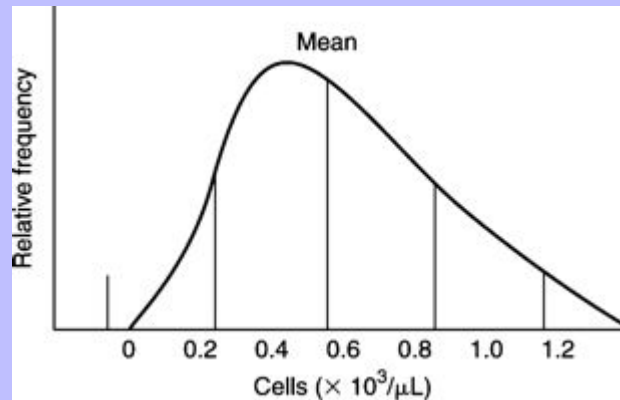
Fig. 1-1



Frequency diagram of a hypothetical plasma analyte with Gaussian distribution. The vertical lines denote standard deviations from the mean. The reference interval calculated by using mean  $\pm$  2 SDs ( $21 \pm 4$  mmol/L) is 17 to 25 mmol/L.

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Fig. 1-2



Frequency diagram of hypothetical absolute blood cell counts with a skewed population. The use of mean  $\pm 2$  SDs to calculate the reference interval is inappropriate, as demonstrated by the lower limit being an impossible negative value.

## 2.2.3 Interpretation of Test Results Relative to Reference Intervals

The common use of the 95% CI to establish reference intervals means that 5% of healthy animals will be reported as having an abnormal result for a given test. When multiple tests are done in laboratory medicine profiles, the probability of at least one test result being abnormal increases with the number of tests done. For example, there is a 64% chance that at least one abnormal test result will be obtained when 20 analytes from a healthy animal are measured. The degree to which a test result is above or below the reference interval is generally important in determining whether a high or low value should be taken seriously. However, some analyte values may increase substantially with minimal cause for alarm (e.g., the serum creatine kinase level can increase several-fold as a result of contamination of the phlebotomy needle with tissue during blood collection), whereas modest changes in other analyte values, such as blood pH or serum potassium concentrations, can be life-threatening.

## 2.2.4 Use of Published Reference Intervals

Routine hematology test results are usually similar among laboratories; consequently, published reference intervals for values such as total leukocyte counts and hematocrits are often used to interpret results from a species (e.g., wallaby) when reference values have not been established in the laboratory conducting the test. Hematology indices such as the red cell distribution width vary more among laboratories, making the use of published reference intervals less acceptable. Some clinical chemistry analytes, such as plasma glucose, vary little among laboratories; but others, such as plasma enzyme activities, vary considerably depending on the methods and instruments used.

The units used in reporting values can vary by laboratory, and a conversion factor may be needed to compare a measured value with a published reference interval. For example, a blood iron value might be reported as 100  $\mu\text{g/dL}$  or 18  $\mu\text{mol/L}$ . Most U.S. laboratories continue to use conventional units, such as milligrams per deciliter ( $\text{mg/dL}$ ), and Canadian and European laboratories use the International System of Units (SI units), such as

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millimoles per liter (mmol/L). When possible, moles are used rather than weight (e.g., milligrams) for SI units. This cannot be done for analytes, such as serum protein concentration, for which the molecular weight is variable or unknown. An SI enzyme unit is defined as 1  $\mu\text{mol}$  of substrate used or product formed per minute. SI units are reported per liter.

For many wild animal species, reference intervals may not be published for some or all tests. The simultaneous measurement of a healthy “control” animal from the same species (when available) can assist in interpretation of the results from the patient.

## 2.3 SENSITIVITY AND SPECIFICITY OF TESTS

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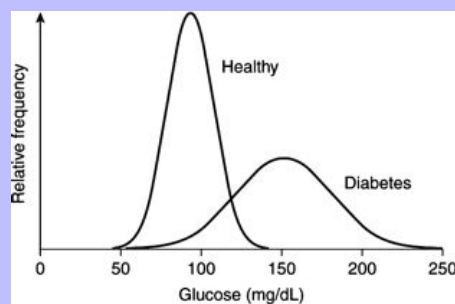
Ideally, analyte values obtained from a healthy animal population would not overlap with values obtained from a diseased animal population. Unfortunately, there is almost always some overlap in the distribution of individual analyte test results between the two groups (Fig. 1-3). When the disease being considered has a major impact on an analyte, little overlap in values will occur; however, extensive overlap occurs if the analyte concentration is minimally altered by the disease being considered. True-positive results refer to the presence of positive test results from animals that have the disease for which they are being tested, and false-positive results refer to the presence of positive test results for animals without the disease for which they are being tested (Fig. 1-4). True-negative results refer to animals with negative test results without the disease for which they are being tested, and false-negative results refer to animals with negative test results that have the disease for which they are being tested. As can be seen in Fig. 1-4, if one increases the reference interval of the healthy population to minimize the number of false-positive results, then the number of false-negative results increases.

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A clinical test should be safe and practical and should accurately indicate the presence or absence of a specific disease or pathologic condition. Sensitivity, specificity, and predictive values constitute measures of a test's utility for confirming or ruling out a given disease.

Sensitivity is the likelihood of a positive or abnormal test result occurring in animals that have the disease being considered (Table 1-1). For example, if 23 of 28 cats with feline infectious peritonitis (FIP) are found to have a low absolute lymphocyte count in blood, the sensitivity of lymphopenia as a diagnostic test for cats with FIP is calculated to be 82% (Table 1-2).

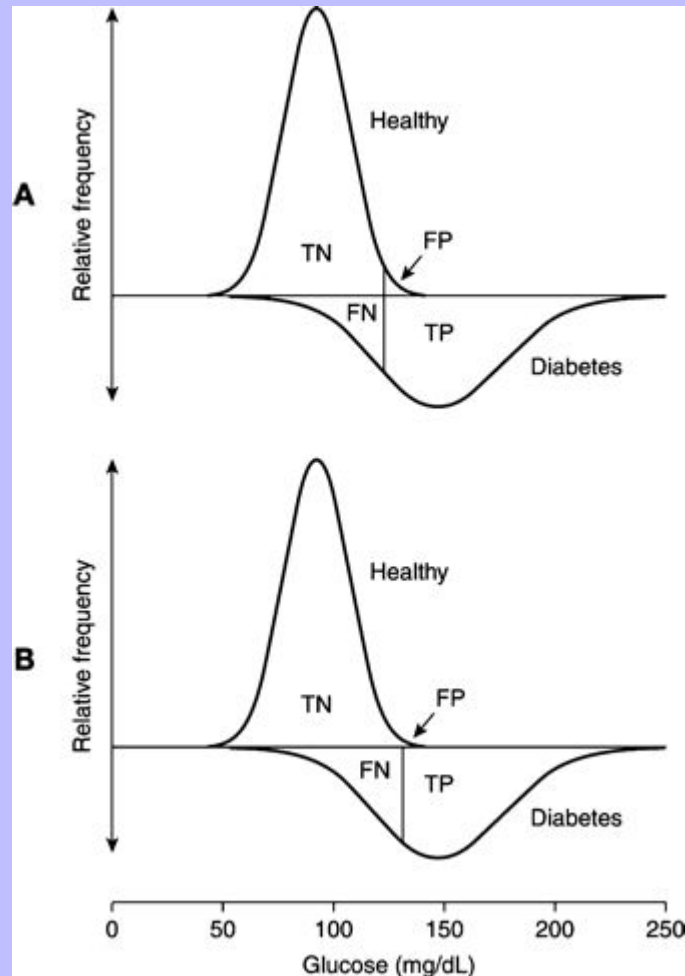
Fig. 1-3



Overlapping Gaussian distributions of a healthy dog population compared with a population of dogs with type 3 diabetes mellitus. (Redrawn from Farver TB: Concepts of normality in clinical biochemistry. In Kaneko JJ, Harvey JW, Bruss ML [eds]: Clinical biochemistry of domestic animals. 5th ed. San Diego, Academic Press, 1997, pp 1-19.)

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Fig. 1-4



Frequency diagrams of a healthy dog population compared with a population of dogs with type 3 diabetes mellitus. Graphs are redrawn from [Figure 1-3](#) to demonstrate true-negative (*TN*), false-negative (*FN*), true-positive (*TP*), and false-positive (*FP*) values used to calculate sensitivity, specificity, and predictive values. The top graph (**A**) demonstrates the effect of using the mean + 2 SDs to set the upper limit of the reference interval. The lower graph (**B**) demonstrates the effect of using the mean + 3 SDs to set the upper limit. The number of FP test results is reduced, but the number of FN test results is increased by using the higher reference limit. (Redrawn from Farver TB: Concepts of normality in clinical biochemistry. In Kaneko JJ, Harvey JW, Bruss ML [eds]: Clinical biochemistry of domestic animals. 5th ed. San Diego, Academic Press, 1997, pp 1-19.)

Specificity is the likelihood of obtaining a negative or normal test result in nondiseased animals (i.e., animals without the particular disease under consideration). In other words, specificity represents the proportion of animals without the disease in question that have normal test results. Specificity may be calculated in two distinctly

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different ways, either by assuming that nondiseased animals consist of only healthy subjects or by assuming that nondiseased animals do not have the particular disease for which the analysis is being performed but may have other diseases.

**Table 1-1 Formulas for the calculation of sensitivity, specificity, predictive value of a positive test, predictive value of a negative test, and prevalence**

Variable	Formula
Sensitivity (%)	$\left( \frac{TP}{TP + FN} \right) \times 100$
Specificity (%)	$\left( \frac{TN}{TN + FP} \right) \times 100$
Predictive value of a positive test (%)	$\left( \frac{TP}{TP + FP} \right) \times 100$
Predictive value of a negative test (%)	$\left( \frac{TN}{TN + FN} \right) \times 100$
Prevalence (%)	$\left( \frac{TP + FN}{TP + TN + FP + FN} \right) \times 100$
TP, True positive (number of animals with the disease for which they are being tested that have a positive test result); FP, false positive (number of animals without the disease for which they are being tested that have a positive test result); TN, true negative (number of animals without the disease for which they are being tested that have a negative test result); and FN, false negative (the number of animals with the disease for which they are being tested that have a negative test result).	

**Table 1-2 Test results from a total of 224 cats with a history and clinical signs, which resulted in feline infectious peritonitis (FIP) being included in the list of differential diagnoses**

Test	Number of Cats Affected	
	With FIP (n = 28)	Without FIP (n = 196)
Lymphopenia ( $<1.5 \times 10^3$ cells/ $\mu$ L)	23	43
Monocytosis ( $>0.9 \times 10^3$ cells/ $\mu$ L)	2	43
Hyperglobulinemia ( $>5.1$ g/dL)	11	7
Coronavirus titer positive	22	84
Data from Sparkes AH, Gruffydd-Jones TJ, Harbour DA: An appraisal of the value of laboratory tests in the diagnosis of feline infectious peritonitis. J Am Anim Hosp Assoc 1994;30:345–350.		

Determining the specificity of a test in a group of healthy animals is of little value because reference intervals are generally established to include 95% of the total population of healthy animals, with 2.5% of healthy animals having values above and 2.5% of healthy animals having values below the reference interval.

The specificity of a test is much more useful when it is determined by using the population of animals typically evaluated in a veterinary hospital setting. In this approach, the “nondiseased” group includes not only healthy animals presented for elective procedures but also animals with diseases other than the disease being considered. Braun et al (2000) demonstrated this difference in specificity when they evaluated increased plasma creatine kinase activity as a diagnostic test for muscle disease. Not surprisingly, the specificity of the test was 98% when calculated by using a group of healthy dogs, but only 73% when calculated by using a group including healthy dogs and ill dogs without muscle disease. The lower specificity occurred in the latter group because plasma creatine kinase activity may also be increased in dogs with nonmuscle diseases.

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## 2.4 PREDICTIVE VALUES AND DISEASE PREVALENCE

Predictive values demonstrate how well a test performs in a given population. In contrast to sensitivity determinations, which are made by using only a population of animals with the disease in question, and specificity determinations, which are made by using only a population of animals without the disease under consideration, predictive value determinations are made from populations that include both animals with and animals without the disease in question.

The predictive value of a positive test (PVPT) concerns only animals in the population being studied that have a positive test result and determines what percentage of animals actually have the disease being considered (see [Table 1-1](#)). It answers the question, “How likely is it that an animal with a positive test result actually has the disease being considered?” In the selected population of cats presented in [Table 1-2](#), there is a  $23/(23 + 43)$  or 35% chance that a cat with lymphopenia in this population has FIP.

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The predictive value of a negative test (PVNT) concerns only animals in the population being studied that have negative or normal test results and determines what percentage of animals with negative test results do not have the disease being considered (see [Table 1-1](#)). It answers the question, “How likely is it that an animal with a negative or normal test result is free of the disease being considered?” In the selected population of cats presented in [Table 1-2](#), there is a  $(196 - 43)/(196 - 43 + 28 - 23)$  or  $153/158$  or 97% chance that a cat with a normal or increased blood lymphocyte count does not have FIP.

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The prevalence of a disease is simply the percentage of animals in a given population that have a certain disease (see [Table 1-1](#)). The prevalence of FIP in the selected population presented in [Table 1-2](#) is  $28/224$  or 12.5%. The prevalence of a disease affects the predictive values of a test used to diagnose the disease, but not its sensitivity or specificity. For most tests, the PVPT will be low and the PVNT will be high if the disease has a low prevalence in the population being studied. The PVPT will be low because low prevalence magnifies the number of false-positive results; that is, most positive test results are false-positive results because few animals actually have the disease (see [Table 1-1](#)). The exception would be a test for which false-positive results essentially do not occur (e.g., polymerase chain reaction tests for specific infectious agents or inherited blood cell defects). The PVNT will be high because few false-negative results are present in a population when the disease prevalence is low.

For the purpose of improving diagnostic accuracy, the prevalence (likelihood) of the disease being considered can be increased by using the history and results of the physical examination and adjunctive diagnostic tests to restrict the population, as described for cats in [Table 1-2](#). The prevalence of FIP in the general cat population is much lower than 12.5%. By ruling out one or more diseases that can produce the same positive test result as the disease being considered, a clinician decreases the size of the population being studied, thereby increasing the prevalence of the disease in the population and increasing the PVPT of the test for the disease being considered, as shown in the example in [Table 1-3](#).

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Table 1-3 Effect of prevalence of disease on the predictive value of a plasma glucose concentration of 128 mg/dL in the diagnosis of diabetes mellitus

Population Studied	Prevalence of Diabetes (%)	Predictive Value of a Positive Test (%)
General dog population	0.4	26
Dogs with history of PU/PD	2	63
Dogs with PU/PD in which renal disease has been ruled out	10	90
Data from MacWilliams PS, Thomas CB: Basic principles of laboratory medicine. Semin Vet Med Surg Small Anim 1992;7:253–261. PU/PD, Polyuria/polydipsia.  A fasting blood glucose of 128 mg/dL or greater was considered to be increased and, therefore, a positive test result.		

Laboratory tests are used to help confirm or rule out a specific diagnosis. When there are significant hazards associated with treatment (e.g., amputation or high-risk chemotherapy) or when euthanasia is being considered, it is necessary to be as certain as possible that the disease is actually present. Consequently, tests with high PVPT are needed for a confirmation strategy. When the penalty for missing a diagnosis is high, such as in the case of a disease for which therapy is effective if begun quickly, tests with high PVNT are theoretically important as a rule-out strategy. A normal test result, by virtue of its high PVNT, would suggest that the disease is not present. Unfortunately, many diseases have low prevalence, which by itself can result in a high PVNT. The best evidence with which to rule out a disease is a negative result of a test that has a high sensitivity for recognizing the disease. In the selected population of cats presented in [Table 1-2](#), finding a normal or increased blood lymphocyte count is more reliable for ruling out FIP than is finding a low lymphocyte count for making a diagnosis of FIP.

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Information is generally available concerning the sensitivity of routine tests for common diseases, but the specificity of a test can vary when populations that include animals with other diseases are analyzed. PVPT and PVNT also vary considerably depending on the population analyzed. Although accurate values are not usually available for PVPT and PVNT, clinicians use their knowledge and experience, combined with the principles outlined previously, to make informed judgments concerning the likelihood that a diagnosis can be confirmed or ruled out. These decisions are seldom based on a single test result; rather, information from the history; clinical signs; and results of physical examination, diagnostic imaging, and other laboratory tests are considered together. The likelihood that a disease is present increases if several findings are supportive of the diagnosis. For example, in the FIP study discussed previously, the PVPT was 35% for cats with lymphopenia; 77% for cats that have lymphopenia and hyperglobulinemia; and 89% for cats that have lymphopenia, hyperglobulinemia, and a positive coronavirus titer. The PVNT increased from 97% when lymphopenia alone was absent to 99% when all three findings were absent. Minimal change occurs in the PVNT because the relatively low disease prevalence in the population is a major contributing factor to the high PVNT. This contribution is most clearly demonstrated by examination of blood monocyte data in the FIP study presented in [Table 1-2](#). Only 7% of cats with FIP have monocytosis (sensitivity) and the PVPT for monocytosis is only 4%, yet the PVNT for a cat lacking monocytosis is 86%.

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Table 1-4 Sensitivity, specificity, and predictive values of plasma fibrinogen concentrations at selected cutoff values for the early identification of foals with *Rhodococcus equi* pneumonia, assuming two different prevalences of disease

Predictive Values			Prevalence 10%		Prevalence 40%	
Cutoff value (mg/dL)	Sensitivity (%)	Specificity (%)	PVPT (%)	PVNT (%)	PVPT (%)	PVNT (%)
300	100	6	11	100	42	100
400	91	51	17	98	55	89
500	71	68	20	96	60	78
600	38	96	51	93	86	70
700	29	97	51	92	86	67
800	12	100	100	91	100	63

Data from Giguere S, Hernandez J, Gaskin J, Miller C, Bowan JL: Evaluation of white blood cell concentration, plasma fibrinogen concentration, and an agar gel immunodiffusion test for the early identification of foals with *Rhodococcus equi* pneumonia. J Am Vet Med Assoc 2003;222:775–781.

PVPT, Predictive value of a positive test; PVNT, predictive value of a negative test.

## 2.5 CUTOFF VALUES

The PVPT may be increased by using a cutoff value above or below the standard reference interval, depending on whether the disease under consideration results in an increase or a decrease in the analyte being measured. For example, low mean cell volume (MCV) is a diagnostic test result suggestive of chronic iron deficiency in dogs. If we use the lower limit of the reference interval (62 fL) to calculate the PVPT, the value would not be remarkably high, because there are various other relatively common disorders that can result in low MCVs in dogs, most notably inflammatory conditions and the presence of portosystemic shunts. However, it is known that the other causes of microcytosis rarely result in MCV values below 52 fL. Consequently, if a dog has an MCV below 52 fL, chronic iron deficiency anemia is highly likely and the PVPT with this cutoff value would approach 100%. However, 52 fL is not routinely used as a cutoff value for a positive test result, because many cases of chronic iron deficiency would be missed. Nonetheless, it is important to realize that dogs with especially low MCV values almost certainly have chronic iron deficiency anemia.

The effects of varying the cutoff value of a test on sensitivity, specificity, and predictive values are shown in [Table 1-4](#), in which plasma fibrinogen concentration is evaluated as a diagnostic test for *Rhodococcus equi* pneumonia in 165 foals from a single farm. It is important to recognize that fibrinogen is an acute-phase protein that often increases in association with various causes of inflammation in horses and that the heat precipitation assay used to measure fibrinogen (although easily performed and clinically useful) is relatively imprecise. As the cutoff value for plasma fibrinogen concentration is increased, the specificity and PVPT increase, but the sensitivity and PVNT decrease (see [Table 1-4](#)). Results from this study also demonstrate that the PVPT increases and the PVNT decreases as the prevalence of disease in a population increases. In choosing the most appropriate cutoff value for a test, one must consider a number of factors including sensitivity and specificity of the test, prevalence of disease in the population being tested, and consequences of false-positive and false-negative test results. In evaluation of the foals, failure to identify an infected foal (false-negative test result) might result in the debilitation or death of the foal. Conversely, the treatment of healthy foals on the basis of false-positive test findings could result in unnecessary financial losses and potential injury to healthy foals as a result of adverse effects of antimicrobial therapy.

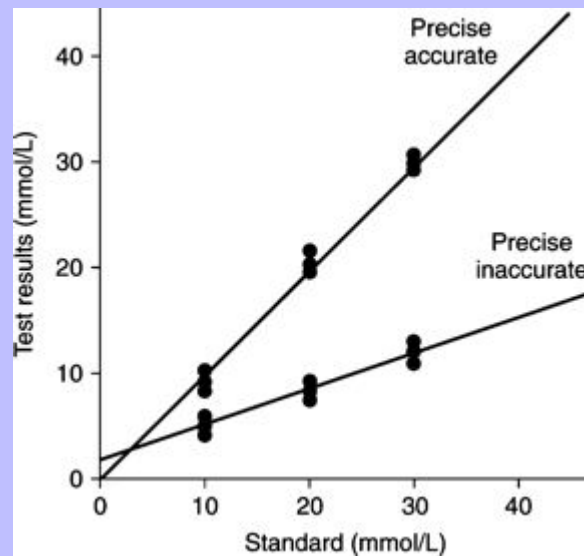
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## 2.6 ACCURACY VERSUS PRECISION

The accuracy of an analytic procedure is determined by how closely the result approaches the true value of the analyte being measured. An accurate test is one in which the average of several results is close to the true value (Figs. 1-5 and 1-6). Analytic procedures with low accuracy are said to have a negative bias if results are below the true value or a positive bias if results are above the true value.

The precision of a test reflects how reproducible the results are when the test is replicated. Precision is independent of accuracy; consequently, test results can be highly reproducible but erroneous (Figs. 1-5 and 1-6). Precision, or more accurately, the amount of imprecision present in a test, is determined by calculating the coefficient of variation (CV) for repeated measurements made on a single sample. The CV is the SD of the repeated measurements expressed as a percentage of the mean of the repeated measurements ( $SD/Mean \times 100$ ).

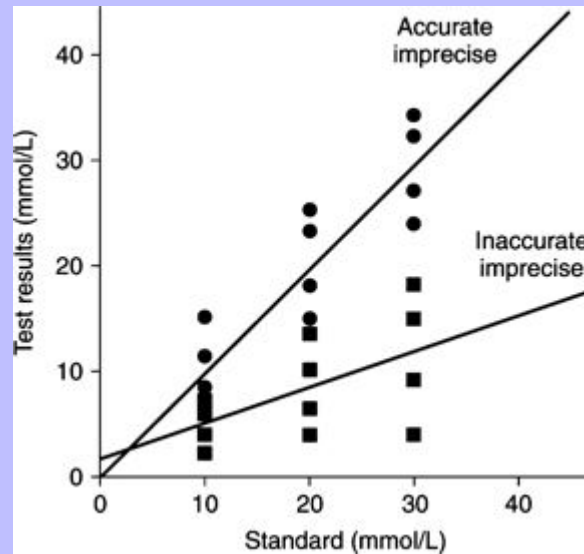
Fig. 1-5



Plots comparing test results of triplicate assays of three standards (*y-axis*) to the known values of the standards (*x-axis*). The top plot is accurate with good precision. The bottom plot has good precision but is inaccurate.

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Fig. 1-6



Plots comparing test results of four replicate assays of three standards (*y*-axis) with the known values of the standards (*x*-axis). The top plot is accurate but imprecise. The bottom plot is inaccurate and imprecise.

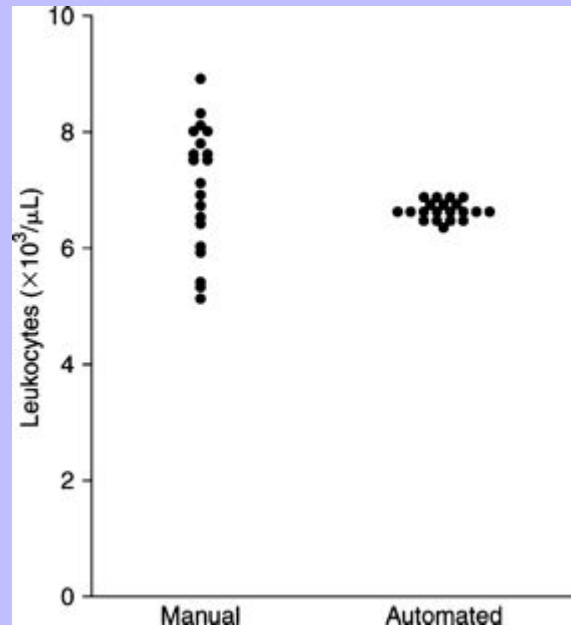
The CV indicates the amount of random error (imprecision) that is present in a test. A high CV (e.g., >10%) indicates that a test lacks precision. A low CV value (e.g., <5%) indicates that test results are reproducible, varying little with repeated measurement. As can be seen in [Figures 1-7](#) and [1-8](#), manual leukocyte counts and manual platelet counts are more imprecise (CVs of 15% and 13%, respectively) than are automated leukocyte counts and automated platelet counts (CVs of 2% and 4%, respectively). These values do not indicate whether manual or automated methods are more accurate. In fact, the mean manual platelet count is probably more accurate (more near the true platelet count) than the mean automated platelet count, because platelets in small platelet clumps can be visualized and counted separately in a hemacytometer chamber, but they would be counted as one platelet or not counted at all in an automated cell counter. The degree of imprecision of a test can also be measured over time intervals to assess variations within runs, between runs, or between days.

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Fig. 1-7



Individual plots of total leukocyte counts performed 20 times each with a manual method and an automated method on the same canine blood sample. For the manual method, 20 separate dilutions in sodium oxalate (Unopette 365855 [Becton Dickinson Co., Franklin Lakes, NJ]) were used, followed by the counting of all leukocytes in 1  $\mu\text{L}$  of 1:100 diluted blood in a hemacytometer chamber. A Cell-Dyn 3500 hematology analyzer (Abbott Laboratories, North Chicago, IL) calibrated for canine blood was used to perform the automated cell counts. The mean and coefficient of variation (CV) for the manual counts were  $7.1 \times 10^3/\mu\text{L}$  and 15%, respectively. The mean and CV for the automated counts were  $6.7 \times 10^3/\mu\text{L}$  and 2%, respectively.

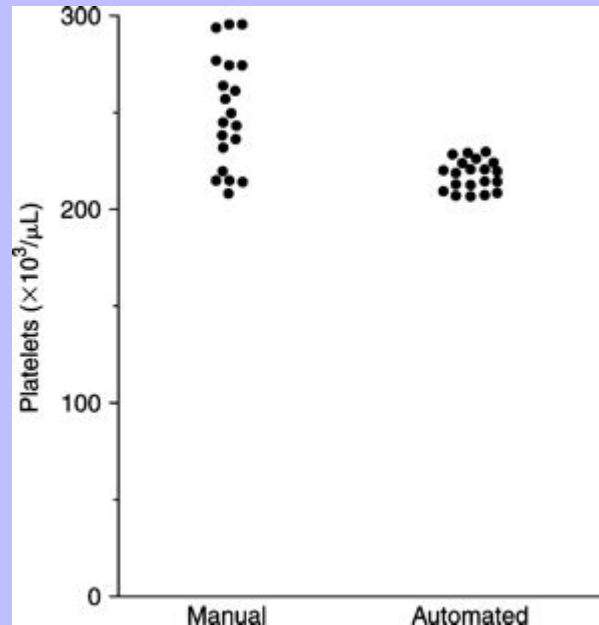
The CV of a test affects how the results are interpreted, especially if a test is being repeated to determine whether a treatment is effective. For example, if the total leukocyte count for a dog is  $4600/\mu\text{L}$  before treatment and  $5800/\mu\text{L}$  after treatment, does this represent a real improvement, or might it reflect imprecision in the measurement of the total leukocyte count? An additional confounding variable in this example is the biologic variability of the animal itself. Jensen et al. (1998) calculated the analytical CV for an automated total leukocyte count in healthy laboratory beagle dogs to be 3.7%, whereas the CV for repeated total leukocyte counts from individual beagles (within-dog CV) was 12.1%. From these numbers, a critical difference of 35% was calculated. This means that the total leukocyte count needs to increase by more than 35% before the therapy can be assumed to have an influence on this analyte. In the example above, the automated total leukocyte count would need to exceed  $4600/\mu\text{L} \times 1.35$  or  $6200/\mu\text{L}$  before a therapeutic effect could be assumed. A considerably greater difference would be required if total leukocyte counts were done by using a manual method because of its higher analytic CV. A greater critical difference might also have been calculated in the above example had client-owned animals been used for this study rather than laboratory animals, because it is likely that the biologic variation would be greater in client-owned animals not accustomed to the phlebotomy procedure, the individuals handling the dogs, or the environment in which the phlebotomy procedure was done.

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Fig. 1-8



Individual plots of platelet counts performed 20 times each with a manual method and an automated method on the same canine blood sample. For the manual method, 20 separate dilutions in sodium oxalate (Unopette 365855 [Becton Dickinson Co., Franklin Lakes, NJ]) were used followed by the counting of all platelets in  $1/25 \mu\text{L}$  of 1:100 diluted blood in a hemacytometer chamber. A Cell-Dyn 3500 hematology analyzer (Abbott Laboratories, North Chicago, IL) calibrated for canine blood was used to perform the automated cell counts.

The mean and coefficient of variation (CV) for the manual counts were  $240 \times 10^3/\mu\text{L}$  and 13%, respectively.

The mean and CV for the automated counts were  $219 \times 10^3/\mu\text{L}$  and 4%, respectively.

Unfortunately, critical difference measurements have been done for few analytes in veterinary medicine, and values will vary depending on methods and instruments used and animal populations evaluated. Nonetheless, clinicians develop knowledge and intuition through study and experience that help them make informed judgments concerning the importance of changes in laboratory data.

### 2.7 QUALITY CONTROL

Commercial control samples, which have been analyzed by reference laboratories, should be run each day, and values obtained should fall within confidence intervals supplied with the control sample. Proficiency testing programs provide external quality control. Samples are periodically sent to participating laboratories for analysis. Results are sent back to the agency supplying the samples, and these values are compared with those from reference laboratories and other participating laboratories that use the same methods. Proficiency testing programs provide valuable peer review of instruments and methods used, but the expense is beyond the means of most private practices.

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## 2.8 ADDITIONAL READING

JP Braun, D Concordet, M Lyazrhi, et al.: Overestimation of the predictive value of positives by the usual calculations of the specificity of diagnostic tests. *Vet Res Commun.* **24**, 2000, 17–24.

Farver, TB: Concepts of normality in clinical biochemistry. In Kaneko, JJ, Harvey, JW, Bruss, ML (Eds.): *Clinical biochemistry of domestic animals*. 5th ed., 1997, Academic Press, San Diego, 1–19.

KP Freeman, Gruenewaldt, J: Quality control validation in veterinary laboratories. *Vet Clin Pathol.* **28**, 1999, 150–155.

S Giguere, J Hernandez, J Gaskin, et al.: Evaluation of white blood cell concentration, plasma fibrinogen concentration, and an agar gel immunodiffusion test for the early identification of foal with *Rhodococcus equi* pneumonia. *J Am Vet Med Assoc.* **222**, 2003, 775–781.

AL Jensen, L Iversen, Petersen, TK: Study on biologic variability of haematological components in dogs. *Comp Haematol Int.* **8**, 1998, 202–204.

Lumsden, JH: “Normal” or reference values: Questions and comments. *Vet Clin Pathol.* **27**, 1998, 102–106.

PS MacWilliams, Thomas, CB: Basic principles of laboratory medicine. *Semin Vet Med Surg (Small Anim)*. **7**, 1992, 253–261.

AH Sparkes, TJ Gruffydd-Jones, Harbour, DA: An appraisal of the value of laboratory tests in the diagnosis of feline infectious peritonitis. *J Am Anim Hosp Assoc.* **30**, 1994, 345–350.

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## <sup>3</sup> Chapter 2 Hematology Procedures

### 3.1 COMPOSITION OF BLOOD

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Blood is composed of cells (erythrocytes, leukocytes, and platelets) circulating within fluid called *plasma* (Fig. 2-1). Erythrocytes or red blood cells are most numerous, with several million erythrocytes per microliter of blood in mammals (see Table 1 in the Appendix). Depending on the species, erythrocytes typically account for one fourth to one half of the total blood volume, as measured by determining the hematocrit. Platelets or thrombocytes are the next most numerous cell type in blood, with platelet counts as low as  $100 \times 10^3/\mu\text{L}$  in healthy horses to several hundred thousand per microliter in other mammalian species. Total leukocyte or white blood cell counts are much lower than erythrocyte and platelet counts, with total leukocyte counts ranging from about  $5 \times 10^3/\mu\text{L}$  to about  $20 \times 10^3/\mu\text{L}$  in mammals. The proportion of leukocyte types present varies by species, with neutrophils being the most numerous leukocyte type present in the blood of carnivores and lymphocytes being the most numerous leukocyte type present in the blood of ruminants.

Plasma consists primarily of water that contains about 6 to 8 g/dL plasma proteins and 1.5 g/dL inorganic salts, lipids, carbohydrates, hormones, and vitamins. Plasma is prepared in the laboratory by collecting blood with an anticoagulant, followed by centrifugation to remove the blood cells. If blood is collected without anticoagulant and allowed to clot, the fluid that is obtained after centrifugation is called *serum*. The protein concentration in serum is usually about 0.2 to 0.5 g/dL lower than that in plasma, primarily because of the absence of fibrinogen in serum, which is consumed during coagulation. Serum proteins may be separated by electrophoresis into albumin,  $\alpha$ -globulins,  $\beta$ -globulins, and  $\gamma$ -globulins. Albumin is a single protein that generally accounts for nearly one half of the total plasma proteins present by weight. Each of the globulin classes is composed of many different proteins, some of which are discussed in Chapter 9.

### 3.2 BLOOD VOLUME CALCULATION

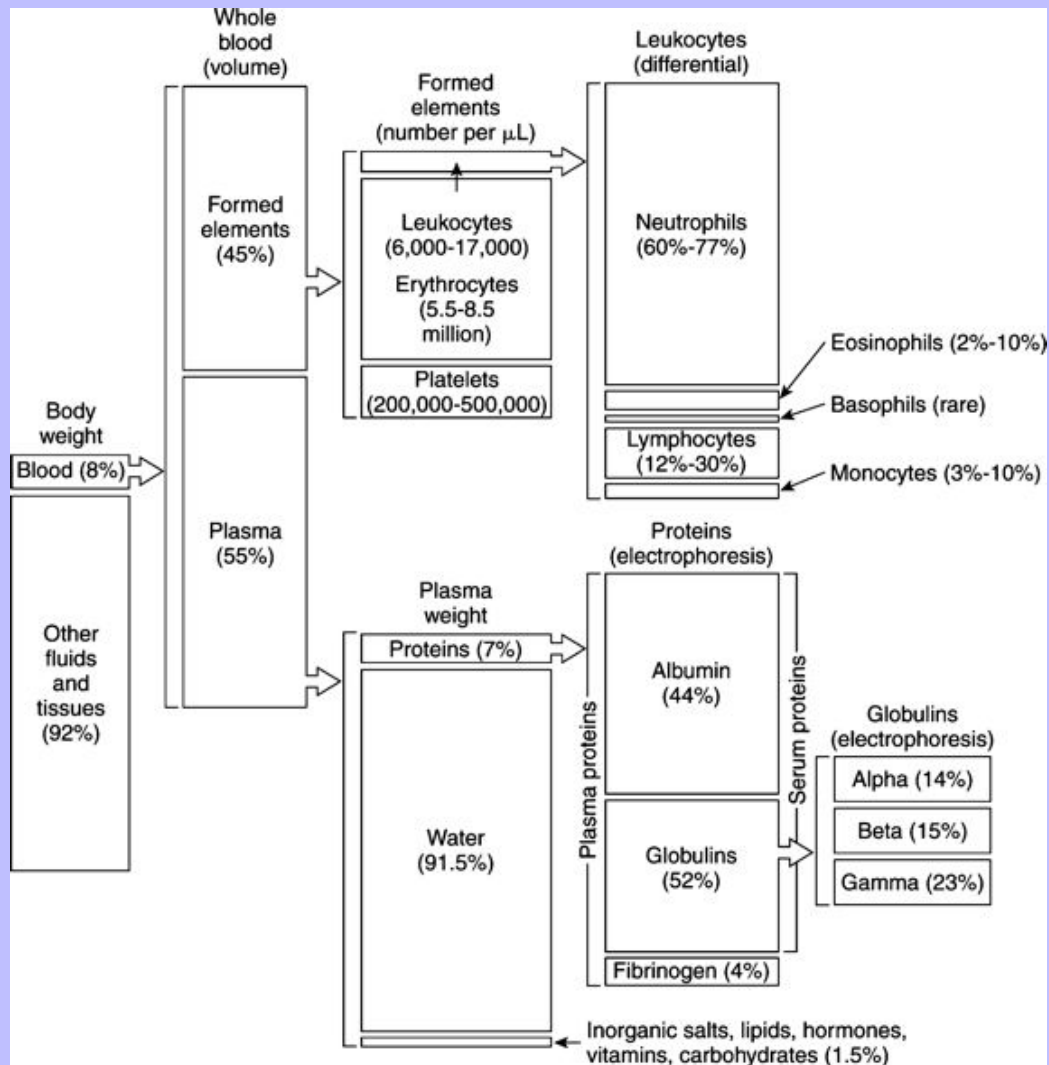
In relation to body weight, total blood volume accounts for about 10% to 11% in hot-blooded horses; 8% to 9% in dogs; 6% to 7% in cats, ruminants, laboratory rodents, and cold-blooded (draft) horses; and 5% to 6% in pigs. The total blood volume of young growing animals often exceeds 10% of body weight. It may be desirable to calculate the total blood volume of an animal when the amount of blood required for transfusion or the amount that can safely be removed for a series of diagnostic tests is being determined or when an animal is to be used as a blood donor. For example, the total blood volume of a 4-kg cat is  $0.07 \times 4 \text{ kg} = 0.28 \text{ kg} = 280 \text{ mL}$ , assuming that 7% of body weight is blood in cats and the specific gravity of blood is 1 (1 mL weighs 1 g). Since one can safely remove 20% of the blood volume from animals, the calculated amount that can be removed from the cat in this example is  $280 \times 0.2 = 56 \text{ mL}$ .

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Fig. 2-1



Approximate composition of blood in the normal adult dog.

## 3.3 SAMPLE COLLECTION AND HANDLING

In monogastric animals, an overnight fast prevents lipemia, which can interfere with plasma protein, fibrinogen, and hemoglobin determinations. Ethylenediaminetetraacetic acid (EDTA) is the preferred anticoagulant for complete blood count determinations in most species, but blood from some birds and reptiles hemolyzes when collected in EDTA. In those species, heparin is often used as the anticoagulant. The disadvantage of using heparin is that leukocytes do not stain as well (presumably because heparin binds to leukocytes) and platelets generally clump more than in blood collected with EDTA.

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Collection directly into a vacuum tube is preferred to collection by syringe and transfer to a vacuum tube, because collection into a vacuum tube reduces platelet clumping and clot formation in samples for complete blood count determinations. Even small clots render a sample unusable. Platelet counts are markedly reduced, and significant reduction can sometimes occur in hematocrit (HCT) and leukocyte counts as well. Also, when the tube is allowed to fill based on the vacuum within the tube, the proper sample-to-anticoagulant ratio will be attained. Inadequate sample size results in decreased HCT because of excess volume of EDTA solution. Care should be taken to avoid iatrogenic hemolysis, which interferes with plasma protein, fibrinogen, and various erythrocyte determinations. Samples should be submitted to the laboratory as rapidly as is feasible, and blood films should be made as soon as possible and rapidly dried to minimize morphologic changes.

## 3.4 GROSS EXAMINATION OF BLOOD SAMPLES

Samples are checked for clots and mixed well (gently inverted 20 times) immediately before aliquots are removed for hematology procedures. Horse erythrocytes settle especially rapidly because of rouleau (adhesion of erythrocytes together like a stack of coins) formation. Gross examination of blood should be done to determine color and evidence of erythrocyte agglutination. The presence of marked lipemia may result in a blood sample with a milky red color that resembles tomato soup when blood is oxygenated.

### 3.4.1 Methemoglobinemia

The presence of large amounts of deoxyhemoglobin accounts for the dark, bluish color of normal venous blood samples. Methemoglobinemia may not be recognized in venous blood samples, because the brownish color of methemoglobin is not readily apparent when mixed with deoxyhemoglobin. When deoxyhemoglobin binds oxygen to form oxyhemoglobin, it becomes bright red; consequently, the brownish coloration of methemoglobin becomes more apparent in the oxygenated samples. A simple spot test provides a rapid way to oxygenate a venous blood sample and determine whether clinically significant levels of methemoglobin are present. One drop of blood from the patient is placed on a piece of absorbent white paper, and a drop of normal control blood is placed next to it. If the methemoglobin content is 10% or greater, the patient's blood will have a noticeably brown coloration, compared with the bright red color of the control blood. Accurate determination of methemoglobin content requires that blood be submitted to a laboratory that offers this test.

### 3.4.2 Agglutination

The appearance of red granules in a well-mixed blood sample suggests the presence of erythrocyte agglutination, the aggregation or clumping of erythrocytes together in clusters. Agglutination is caused by the presence of immunoglobulins bound to erythrocyte surfaces. It must be differentiated from rouleau formation that results from the adhesion of erythrocytes together like a stack of coins, as can be seen in blood from healthy horses and cats. Agglutination can be differentiated from rouleau by washing erythrocytes in physiologic saline solution or adding 4 drops of physiological saline solution to a drop of anticoagulated blood to see if the aggregation of erythrocytes is dispersed (rouleau) or remains (agglutination).

### 3.4.3 Microhematocrit Tube

A microhematocrit tube is filled to about 90% of capacity with well-mixed blood and sealed with clay at one end. The tube is then placed in a microhematocrit centrifuge with the clay plug oriented to the periphery of the centrifuge head and centrifuged for 5 minutes. After centrifugation, the blood sample will be separated into three

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layers based on density, with packed erythrocytes located at the bottom. A white “buffy coat” is located above the erythrocyte layer, and the acellular plasma is located above the buffy coat. The HCT is measured by determining the fraction of total blood volume occupied by erythrocytes in the tube. Leukocytes and platelets are primarily located within the buffy coat, although certain leukocyte types may be present in the top portion of the packed erythrocyte column in some species (e.g., neutrophils in cattle). The width of the buffy coat generally correlates directly with the total leukocyte count. A large buffy coat suggests leukocytosis or thrombocytosis, and a small buffy coat suggests that low numbers of these cells may be present. The buffy coat may appear reddish because of the presence of a marked reticulocytosis.

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The plasma in the microhematocrit tube is normally clear and colorless in dogs, cats, pigs, and sheep. It is clear and light yellow in horses because the plasma bilirubin concentration is higher in horses than that in other domestic animal species. Plasma may appear light yellow in cattle that eat green plants containing carotenoids. Increased yellow coloration of plasma usually indicates increased bilirubin concentrations. This often occurs in association with anorexia (fasting hyperbilirubinemia) in horses as a result of reduced removal of unconjugated bilirubin by the liver. In other species, yellow plasma with a normal HCT suggests hyperbilirubinemia caused by liver disease. Hyperbilirubinemia associated with a marked decrease in the HCT suggests increased destruction of erythrocytes.

Lipemia is identified by a white opaque appearance of plasma that is caused by increased concentrations of chylomicrons and/or very low density lipoproteins. The presence of chylomicrons may also result in a white layer at the top of the plasma column. Lipemia is most often the result of a recent meal (postprandial lipemia), but diseases including diabetes mellitus, pancreatitis, and hypothyroidism may cause lipemia in dogs. Hereditary causes include lipoprotein lipase deficiency in cats and dogs and idiopathic hyperlipidemia in miniature schnauzers. Ponies (especially obese ones), miniature horses, and donkeys are susceptible to lipemia associated with pregnancy, lactation, and/or anorexia. These conditions result in the mobilization of unesterified fatty acids from adipose tissue and subsequent overproduction of very low density lipoproteins by the liver.

The presence of hemoglobin is indicated if the plasma appears red. The hemoglobin in plasma can result from the lysis of red blood cells during the collection or handling of blood samples or from true intravascular hemolysis. Plasma will also appear red after treatment with blood substitute cross-linked hemoglobin solutions such as Oxyglobin (Biopure Corporation, Cambridge, MA). Some in vitro hemolysis is often observed in lipemic samples. The presence of hemoglobinuria indicates that intravascular hemolysis has occurred.

After the HCT is measured and the appearances of the plasma and the buffy coat are noted, the microhematocrit capillary tube is broken just above the buffy coat and the plasma is placed in a refractometer for plasma protein determination. Plasma protein values in newborn animals (approximately 4.5 to 5.5 g/dL) are lower than adult values and increase to the adult range by 3 to 4 months of age. The presence of lipemia or hemolysis will falsely increase the measured plasma protein value.

Fibrinogen can also be measured in a microhematocrit tube because it readily precipitates from plasma when heated to 56°C to 58°C for 3 minutes. The difference between the total protein of the plasma and the total protein of the defibrinogenated (heated) plasma provides an estimate of the fibrinogen concentration in the plasma. This method is useful for detecting high fibrinogen concentrations, but it is not accurate for detecting low fibrinogen concentrations.

## 3.5 BLOOD CELL COUNTING AND SIZING

Total leukocyte counts, erythrocyte counts, and platelet counts may be determined by using manual or automated techniques in mammals.

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## 3.5.1 Manual Cell Counting

Manual erythrocyte counts are not accurate enough to be useful. Manual total leukocyte counts and platelet counts can be performed with the Unopette 5850 reservoir and pipette (Becton Dickinson, Franklin Lakes, NJ) to dilute the sample and lyse erythrocytes before microscopic counting of leukocytes and platelets in a hemacytometer chamber. Manual leukocyte counts and platelet counts are done when errors are suspected in cell counts generated by automated cell counters. Manual cell counts may also be done in an emergency situation when automated cell counters are not available.

All blood cell types in birds and reptiles are nucleated, making accurate separation and counting difficult or impossible in automated cell counters. Consequently, manual leukocyte counts are generally required for nonmammalian species. Thrombocytes can be estimated on the basis of the number present in stained blood films. The reader is referred to the article by Pierson in the reference list for the manual methods used. 17  
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If properly maintained, automated blood cell counters are more precise and accurate than manual counting for mammalian species. Various technologies including quantitative buffy coat analysis, impedance measurements, laser flow cytometry, and cytochemistry are used to generate these cell counts.

## 3.5.2 Automated Cell Counting

Quantitative buffy coat analysis (QBC VetAutoread Hematology System; IDEXX, Inc., Westbrook, ME) depends on variations in cell density to separate cell types. Cells are not actually counted, but rather the width of the various layers of cell types that form are measured and the cell “count” is generated, assuming a standard cell size for the cell type in question.

Impedance counters such as the Coulter particle counters (Model S series and Model ZBI, Coulter Corporation, Miami, FL) operate on the principle that cells are poor electrical conductors. Blood is diluted in an electrically conductive solution, and a precise volume of this diluted suspension is drawn through a small aperture between two electrodes. Each cell produces a change in electrical impedance, resulting in a change in voltage that is proportional to the size of the cell counted. Several thousand cells can be counted and sized per second. Erythrocytes and platelets can be differentiated by size alone in many species by using impedance counters, but not in cats, in which the platelets are large and erythrocytes are relatively small. Leukocytes are counted as free nuclei after lysis of erythrocytes and platelets. Nucleated erythrocytes are counted as leukocytes in impedance counters; consequently, total leukocyte counts may need to be corrected for the presence of nucleated erythrocytes.

New advanced automated cell counters, such as the Cell-Dyn 3500 hematology analyzer (Abbott Laboratories, North Chicago, IL) and the Advia 120 (Bayer Diagnostics, Tarrytown, NY) use laser flow cytometry. Individual cells pass through a laser beam, absorbing and scattering light. Interruptions in light are used to count cells, and light scatter is used to determine size and internal complexity or density. The Advia 120 also uses a peroxidase channel to aid in determining specific leukocyte types. Differences in size, complexity, and cytochemical staining (Advia 120) allow for the determination of five-part differential leukocyte counts, although automated basophil counts in animals require validation. Although the Cell-Dyn 3500 hematology analyzer uses laser flow technology for leukocyte differential counts, it still uses impedance technology for erythrocyte and platelet counting and sizing.

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## 3.5.3 Errors in Blood Cell Measurements

The accuracy of blood cell counting depends on the quality and characteristics of the blood sample, as well as the accuracy of the analytic methods used. Storage of blood samples for more than a few hours can result in sample deterioration and inaccurate cell counts. The presence of even small clots in the blood tube invalidates all cell counts. Even without clot formation, platelet aggregation may occur if platelets become activated during blood collection, which may occur when blood is obtained with a syringe and then transferred to an anticoagulant tube. Heparin is generally not used as an anticoagulant because it does not prevent platelet clumping and it is associated with poor leukocyte staining on blood films. EDTA is the preferred anticoagulant for complete blood count determinations in most species, but platelet and leukocyte aggregates may occur in some samples. Collection of blood into citrate may prevent this problem. Cell aggregation tends to be more pronounced as blood is cooled and stored; consequently, processing samples as rapidly as possible after collection may minimize the formation of leukocyte and platelet aggregates. EDTA can cause marked hemolysis in blood from some species of birds (ostriches, emus, and jays) and some reptiles (turtles and tortoises). Heparin is used as an anticoagulant in blood samples from these bird and reptile species. Both lysis and clumping can result in reduced counts of the cell types involved.

The presence of severe lipemia can result in spuriously increased hemoglobin and mean cell hemoglobin concentration (MCHC) values, and possibly even an increased platelet count. The presence of in vitro or in vivo hemolysis in the sample or prior treatment with a cross-linked hemoglobin solution can result in an erroneously increased MCHC value. The presence of numerous Heinz bodies in erythrocytes can result in spuriously increased hemoglobin and MCHC values, and sometimes, increased total leukocyte counts.

Not only can platelet clumping result in an erroneously decreased platelet count, it can also result in a falsely increased mean platelet volume and, occasionally, a falsely increased total leukocyte count. Agglutination of erythrocytes in a blood sample can result in a spuriously increased mean cell volume and decreased HCT in addition to a reduced total erythrocyte count. As indicated earlier, the presence of nucleated erythrocytes can result in a falsely increased total leukocyte count in some hematology analyzers. Residual erythrocyte stroma from inadequate lysing of erythrocytes may also result in spuriously increased total leukocyte counts.

Laboratory errors may occur as a result of mistakes made by operators. These operator errors include lack of knowledge or skills related to the test being done, improper labeling of samples, dilution errors, use of outdated reagents, inadequate quality control measures, and improper calibration of equipment. The instruments being used must be optimally calibrated and validated for the species being tested.

Erythrocytes and platelets vary considerably in volume among animal species, and instruments must be adjustable to accurately count and size these blood cells for the species being evaluated. Cats naturally have large platelets and moderately small erythrocytes. The resultant overlap of erythrocyte and platelet size makes separation of cat platelets and erythrocytes unreliable when impedance counters are used. Consequently, cat platelet counts are spuriously decreased when measured with impedance counters. The inclusion of platelets in erythrocyte measurements can result in increased erythrocyte counts and HCT values and reduced mean cell volume and MCHC values, but the ratio of platelets to erythrocytes is usually not large enough to have appreciable effects on these values. Exceptions include cats with severe anemia, marked thrombocytosis, or both. Leukocytes are generally included in erythrocyte measurements, but the ratio of leukocytes to erythrocytes is usually not large enough to have appreciable effects on erythrocyte numbers. Exceptions include animals with severe anemia and marked leukocytosis. In these instances, the inclusion of leukocytes in erythrocyte measurements can result in increased erythrocyte counts and HCT and mean cell volume values and reduced MCHC values, because leukocytes are larger than erythrocytes. These errors, which result from difficulties in

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separating cell types by size alone, should be minimized in automated cell counters that separate cells not only by size but also by internal complexity.

With the advent of new laser flow cytometry techniques, it is now possible to perform automated differential leukocyte counts; however, the ability to accurately count basophils remains to be demonstrated in domestic animals. These flow cytometers must be specifically calibrated for each species. They work best when leukocyte structure is normal. More reliable flags are needed to identify the presence of left shifts and neoplastic cells.

The examination of a stained blood film is essential as a quality control measure, regardless of the technology used to count blood cells. In addition to verifying the accuracy of leukocyte and platelet counts, a number of other evaluations are made when a blood film is examined. Examples include determination of whether erythrocyte polychromasia, erythrocyte shape abnormalities, neutrophilic left shifts, neutrophil toxicity, reactive lymphocytes, blast cells, mast cells, or blood parasites are present.

## 3.6 BLOOD FILM PREPARATION

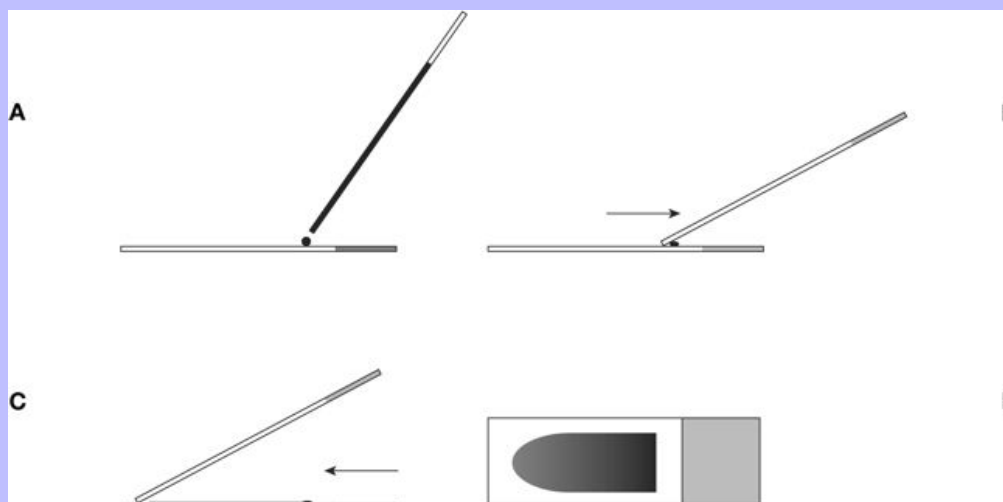
Blood films should be prepared within a couple of hours of blood sample collection to avoid artifactual changes that will distort the structure of blood cells. Blood films are prepared in various ways, including the slide (wedge) method, coverslip method, and automated slide spinner method. It is essential that a monolayer of intact cells be present on the slide so that accurate examination and differential leukocyte counts can be performed.

### 3.6.1 Slide Blood Film Method

A clean glass slide is placed on a flat surface, and a small drop of well-mixed blood is placed on one end of the slide by using a microhematocrit tube (Fig. 2-2, A). This slide is held in place with one hand, and a second glass slide (spreader slide) is placed on the first slide and held between the thumb and forefinger with the other hand at an angle of about 30 degrees in front of the drop of blood. The spreader slide is then backed into the drop of blood (Fig. 2-2, B), and as soon as the blood flows along the back side of the spreader slide, the spreader slide is rapidly pushed forward (Fig. 2-2, C). The thickness of the smear is influenced by the viscosity of the sample. Consequently, the angle between the two slides may be increased when the blood is less viscous (low HCT) and decreased when the blood is more viscous (high HCT) than normal to produce a smear of appropriate thickness.

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Fig. 2-2



Slide blood film preparation. **A**, A glass slide is placed on a flat surface and a small drop of well-mixed blood is placed on one end of the slide by using a microhematocrit tube. **B**, A second glass slide (spreader slide) is placed on the first slide at an angle of about 30 degrees in front of the drop of blood. The spreader slide is then backed into the drop of blood. **C**, As soon as the blood flows along the back side of the spreader slide, the spreader slide is rapidly pushed forward. **D**, The blood film produced is thick at the back of the slide, where the drop of blood was placed, and thin at the front (feathered) edge of the slide.

If the drop of blood is the proper size, all blood will remain on the slide as the spreader slide is advanced, and a smear will be prepared that is thick at the back of the slide, where the drop of blood was placed, and thin at the front (feathered) edge of the slide (Fig. 2-2, **D**). If the drop of blood is too large, some of the blood will be pushed off the end of the slide, causing potential problems. Often these blood films will be too thick for accurate evaluation. In addition, clumps of cells tend to be pushed off the slide, making them unavailable for examination.

Once prepared, the slide is immediately dried by waving it in the air or by holding it in front of a hair dryer set on a warm air setting. Holding the slide close to a dryer set on a hot air setting can result in fragmentation of cells. Each slide is identified by writing on the thick end of the blood film or the frosted end of the slide with a graphite pencil or on the frosted or unfrosted end of the slide with a felt-tipped marker containing ink that is not removed by alcohol fixation.

### 3.6.2

#### Coverslip Blood Film Method

Two 22-mm square No. 1½ coverslips are required. A camel's hair brush is used to remove particles from the surfaces that will have contact with blood. One coverslip is held between the thumb and index finger of one hand, and a small drop of blood is placed in the middle of it by using a microhematocrit tube (Fig. 2-3, **A**). The drop of blood should be as perfectly round as possible to produce even spreading between coverslips (Fig. 2-3, **B**). The second coverslip is dropped on top of the first in a crosswise position (Fig. 2-3, **C**). After the blood spreads evenly between the two coverslips and a feathered edge forms at the periphery (Fig. 2-3, **D**), the coverslips are rapidly separated by grasping an exposed corner of the top coverslip with the other hand and

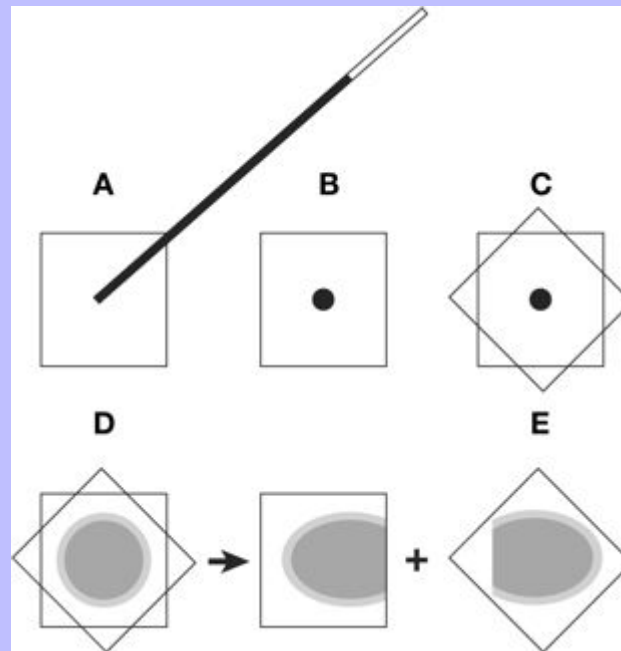
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pulling them apart in a smooth, horizontal manner (Fig 2-3, E). Coverslips are immediately dried as described previously and then identified by marking on the thick end of the blood films with a graphite pencil or on the uncovered glass surfaces of the coverslip with a felt-tipped marker containing ink that is not removed by alcohol fixation. If the drop of blood used is too large, a feathered edge will not form and the blood film will be too thick. Multiple coverslip blood films may be stained in small slotted Coplin jars or in ceramic staining baskets placed in beakers of fixative and stain.

Fig. 2-3



Coverslip blood film preparation. **A and B**, One clean coverslip is held between the thumb and index finger of one hand and a small drop of blood is placed in the middle of it by using a microhematocrit tube. **C**, A second clean coverslip is dropped on top of the first in a crosswise position. **D**, Blood spreads evenly between the two coverslips and a feathered edge forms at the periphery. **E**, The coverslips are rapidly separated by grasping an exposed corner of the top coverslip with the other hand and pulling them apart in a smooth, horizontal manner.

### 3.7 BLOOD FILM STAINING PROCEDURES

#### 3.7.1 Romanowsky-Type Stains

Blood films are routinely stained with a Romanowsky-type stain (e.g., Wright or Wright-Giemsa stain) either manually or by using an automatic slide stainer. Romanowsky-type stains are composed of a mixture of eosin and oxidized methylene blue (azure) dyes. The azure dyes stain acids, resulting in blue to purple colors; and eosin stains bases, resulting in red coloration (Plates 1, 2 to 345, and 7 to 89101112131415161718). These staining characteristics depend on the pH of the stains and rinse water, as well as the nature of the cells present. Pale-staining cells can result from inadequate staining time, degraded stains, or excessive washing.

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Blood films may have an overall blue tint if they are stored unfixed for weeks before staining or if the unfixed blood films are exposed to formalin vapors, as occurs when blood films are shipped to the laboratory in a package that also contains formalin-fixed tissue. Blood films prepared from blood collected with heparin as the anticoagulant have an overall magenta tint because of the mucopolysaccharides present.

Various problems that result in poor-quality films can occur during drying, fixation, and staining of blood films. Drying or fixation problems can result in variably shaped refractile inclusions in erythrocytes, which may be confused with erythrocyte parasites. The presence of stain precipitation can make identification of leukocytes and blood parasites difficult. Precipitated stain may be present because the stain needed to be filtered, the staining procedure was too long, or washing was not sufficient. In an attempt to prevent abdominal adhesions, carboxymethylcellulose has been infused intraperitoneally in horses and cattle. After absorption into blood, the carboxymethylcellulose precipitate must be differentiated from stain precipitation.

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Various rapid stains are available; however, the quality of stained blood films is generally somewhat lower than that obtained with longer staining procedures. The Diff-Quik stain is a commonly used Romanowsky-type rapid blood stain. The quality of this staining procedure is improved considerably by allowing the blood film to remain in the fixative for several minutes. One limitation of this stain is that it does not stain basophil or mast cell granules well. However, it is superior to Wright and Wright-Giemsa stains in staining distemper inclusions in canine blood cells.

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## 3.7.2 Reticulocyte Stains

Reticulocyte stains are commercially available. Those wishing to prepare their own stain can do so by dissolving 0.5 g of new methylene blue and 1.6 g of potassium oxalate in 100 mL of distilled water. After filtration of the stain, equal volumes of blood and stain are mixed together in a test tube and incubated at room temperature for 10 to 20 minutes. After incubation, blood films are made and reticulocyte counts are done by examining 1000 erythrocytes and determining the percentage that are reticulocytes. The use of a Miller's disk in one of the microscope oculars saves time in performing the reticulocyte count.

The blue-staining aggregates, or “reticulum,” seen in reticulocytes (Plate 2) do not occur as such in living cells but result from the precipitation of ribosomal RNA in immature erythrocytes during the staining process. This RNA also causes the bluish discoloration in polychromatophilic erythrocytes stained with Romanowsky-type stains. As a reticulocyte matures, the number of ribosomes decreases until only small punctate (dot-like) inclusions are observed in erythrocytes (punctate reticulocytes) stained with the reticulocyte stain. The cell in question should have two or more discrete blue granules that are visible without requiring fine-focus adjustment to reduce the chance that a staining artifact would result in misclassification of a mature erythrocyte as a punctate reticulocyte when a reticulocyte stain is used. These inclusions should be away from the cell margin to avoid confusion with hemotropic mycoplasmas (formerly *Haemobartonella* organisms) or small Heinz bodies.

In healthy cats, as well as in cats with regenerative anemia, the number of punctate reticulocytes is much greater than that seen in other species. This apparently occurs because the loss of ribosomes during the maturation of reticulocytes in cats is slower than that in other species. Consequently, reticulocytes in cats are classified as aggregate (if coarse clumping is observed) or punctate (if small individual inclusions are present). Percentages of both types should be reported.

In contrast to those of the cat, most reticulocytes in other species are of the aggregate type. Consequently, no attempts are made to differentiate stages of reticulocytes in species other than the cat. The percentage of

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reticulocytes in most species correlates directly with the percentage of polychromatophilic erythrocytes observed on routinely stained blood films.

Heinz bodies are composed of denatured, precipitated hemoglobin. They are spherical, stain pale blue with reticulocyte stains, and are usually found at the periphery of the erythrocyte.

### 3.7.3 New Methylene Blue “Wet Mounts”

A new methylene blue “wet mount” preparation can be used to rapidly obtain information concerning the number of reticulocytes, platelets, and Heinz bodies present. The stain consists of 0.5% new methylene blue dissolved in 0.85% saline solution. One milliliter of formalin is added per 100 mL of stain as a preservative. This stain is filtered after preparation and stored in dropper bottles. Alternatively, the stain may be stored in a plastic syringe with a 0.2- $\mu$ m syringe filter attached so that it is filtered as it is used. Dry, unfixed blood films are stained by placing a drop of stain between the coverslip and glass slide. This preparation is not permanent and does not stain mature erythrocytes or eosinophil granules. It does not demonstrate punctate reticulocytes, but aggregate reticulocytes appear as erythrocyte ghosts containing blue to purple granular material (Fig. 2-4). Platelets stain blue to purple, and Heinz bodies appear as refractile inclusions within erythrocyte ghosts. Although this staining method is not optimal for differential leukocyte counts, the number and type of leukocytes present can be appreciated.

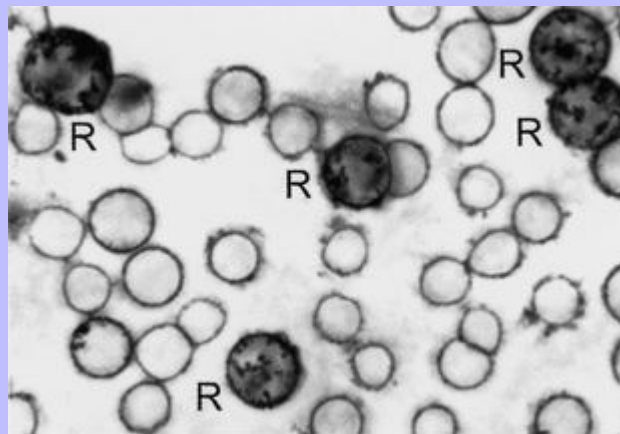
### 3.7.4 Iron Stain

An iron stain, such as the Prussian blue stain, is used to verify the presence of iron-containing (siderotic) inclusions in blood and bone marrow cells and to evaluate bone marrow iron stores. Smears may be sent to a commercial laboratory for this stain, or a stain kit can be purchased and applied in-house (Harleco Ferric Iron Histochemical Reaction Set, No. 6498693; EM Diagnostic Systems, Gibbstown, NJ). Iron-positive material stains blue in contrast to the pink color of the cells and background when this stain is applied.

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Fig. 2-4



Reticulocytes (R) in blood from a dog with a regenerative anemia in a new methylene blue–stained wet preparation.

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The presence of focal areas of basophilic stippling within erythrocytes stained with Romanowsky-type blood stains suggests that the stippling may contain iron. Iron-containing erythrocytes are referred to as *siderocytes*. Neutrophils and monocytes may contain dark bluish-black or greenish iron-positive particles within their cytoplasm when stained with Romanowsky-type stains. Leukocytes containing iron-positive inclusions have been called *sideroleukocytes*.

Application of Prussian blue stain to bone marrow aspirate smears is a useful way to evaluate the amount of storage iron present in the marrow. Minimal or no iron is expected in iron deficiency anemia (although cats normally have no stainable iron in the marrow), whereas normal or excess iron may be observed in animals with hemolytic anemia and animals with anemia resulting from decreased erythrocyte production.

## 3.7.5 Cytochemical Stains

A variety of cytochemical stains (e.g., peroxidase, chloracetate esterase, alkaline phosphatase, nonspecific esterase) are used to classify cells in animals with acute myeloid leukemia. Reactions vary not only by cell type and stage of maturation but also by species. These stains are done in a limited number of laboratories, and special training and/or experience is required to interpret the results.

## 3.8 EXAMINATION OF STAINED BLOOD FILMS

An overview and organized method of blood film examination are presented in this chapter. Descriptions and photographs of normal and abnormal blood cell structure, inclusions, and infectious agents are provided in subsequent chapters.

Blood films are generally examined after staining with Romanowsky-type stains such as Wright or Wright-Giemsa stain. These stains allow for examination of erythrocyte, leukocyte, and platelet structure. Blood films should first be scanned by using a low-power objective to estimate the total leukocyte count and to check for the presence of erythrocyte agglutination, leukocyte aggregates, platelet aggregates, microfilaria, and abnormal cells that might be missed during the differential leukocyte count. It is particularly important that the feathered end of blood films made on glass slides be examined because leukocytes and platelet aggregates may be concentrated in this area. Aggregates of cells tend to be in the center of coverslip blood films rather than at the feathered edge.

When a glass slide blood film is examined, the film will be too thick to evaluate blood cell structure at the back of the slide where the drop of blood was applied and too thin at the feathered edge where cells are distorted. The optimal area for evaluation is generally in the front half of the smear behind the feathered edge. This area should appear as a well-stained monolayer of cells. A monolayer is defined as a field in which erythrocytes are close together, with approximately one half of the erythrocytes touching each other.

### 3.8.1 Leukocyte Evaluation

As a quality control measure, the number of leukocytes present should be estimated to ensure that the number present on the slide is consistent with the total leukocyte count measured. If 10× oculars and a 10× objective are used (100× magnification), the total leukocyte count in blood (cells per microliter) may be estimated by determining the average number of leukocytes present per field and multiplying by 125. If a 20× objective is used, the total leukocyte count may be estimated by multiplying the average number of leukocytes per field by 500. The correction factor used may vary, depending on the microscope used. Consequently, one should

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determine the appropriate correction factors for the microscope being used by performing estimates on a number of blood films on which the total leukocyte counts have been accurately determined in blood.

A differential leukocyte count is done by identifying 200 consecutive leukocytes (see [Plates 9 to 101112131415](#)) with the use of a 40× or 50× objective. Because neutrophils tend to be pulled to the edges in a wedge-type (glass slide) blood smear and lymphocytes tend to remain in the body of the smear, differential counts are done by examining cells in a pattern that evaluates both the edges and the center of the smear. After the count is complete, the percent of each leukocyte type present is calculated and multiplied by the total leukocyte count to get the absolute number of each cell type present per microliter of blood. It is the absolute number of each leukocyte type that is important. Relative values (percentages) can be misleading when the total leukocyte count is abnormal. Let us consider two dogs, one with 7% lymphocytes and a total leukocyte count of 40,000/μL and the other with 70% lymphocytes and a total leukocyte count of 4000/μL. The first dog would be said to have a “relative” lymphopenia and the second dog would be said to have a “relative” lymphocytosis, but they both have the same normal absolute lymphocyte count (2800/μL).

Nucleated red blood cells (NRBCs) are counted along with leukocytes when leukocyte counts are done using manual methods or automated impedance cell counters. When encountered during blood film examination, the number of NRBCs per 100 leukocytes should be tabulated and the total leukocyte count should be corrected for the number of NRBCs present (see formula below) before calculating the absolute cell counts for each blood cell type.

Corrected leukocyte count  
= (measured leukocyte × 100)/(100 + NRBC)

Table 2-1 Semiquantitative evaluation of toxic changes in the cytoplasm of neutrophils

Morphologic change	Relative severity
Döhle bodies*	1+
Mildly basophilic	1+
Mildly basophilic with Döhle bodies	2+
Mildly basophilic and foamy	2+
Dark blue-grey and foamy†	3+
Basophilic with toxic granules†	3+

\* Döhle bodies are sometimes seen in neutrophils of cats that do not exhibit signs of illness.

† May also contain Döhle bodies.

If leukocyte counts are done using automated cell counters that use technology which can differentiate NRBCs from leukocytes (e.g., morphologic characteristics of cells are determined using a laser beam), the above calculation will not be necessary. However, the number of NRBCs present should still be recorded.

The presence of abnormal leukocyte structure, such as toxic cytoplasm in neutrophils or increased reactive lymphocytes (e.g., more than 5% of lymphocytes are reactive), should be recorded on the hematology report form. The frequency of toxic neutrophils is reported as few (5%–10%), moderate (11%–30%), or many (>30%), and the severity of toxic change is recorded as 1+ to 3+ ([Table 2-1](#)).

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## 3.8.2 Erythrocyte Morphology

Erythrocyte morphology should be examined and recorded as either normal or abnormal (see [Plates 1, 3 to 45](#)). Erythrocytes on blood films from healthy horses, cats, and pigs often exhibit rouleau formation, and erythrocytes from horses and cats may contain a low percentage of small, spherical nuclear remnants called *Howell-Jolly bodies*. Rouleau formation and the presence of Howell-Jolly bodies ([Plate 1](#)) should be recorded on the hematology form when present on blood films from species in which these are not normal findings.

Additional observations regarding erythrocyte structure, such as the degree of polychromasia (presence of polychromatophilic erythrocytes), anisocytosis (variation in size), and poikilocytosis (abnormal shapes) should be made. Polychromatophilic erythrocytes are reticulocytes that stain bluish-red because of the combined presence of hemoglobin (red-staining) and ribosomes (blue-staining). Abnormal erythrocyte shapes should be classified as specifically as possible, because specific shape abnormalities can help determine the nature of a disorder that may be present. Examples of abnormal erythrocyte structure include echinocytes, acanthocytes, schistocytes, keratocytes, dacryocytes, elliptocytes, eccentrocytes, and spherocytes. The number of abnormal cells should be reported in a semi-quantitative fashion such as that shown in [Table 2-2](#).

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**Table 2-2 Semiquantitative evaluation of erythrocyte morphology based on the average number of abnormal cells per 1000× microscopic monolayer field\***

Morphology	Magnitude of occurrence			
	1+	2+	3+	4+
Anisocytosis				
Dog	7–15	16–20	21–29	>30
Cat	5–8	9–15	16–20	>20
Cattle	10–20	21–30	31–40	>40
Horse	1–3	4–6	7–10	>10
Polychromasia				
Dog	2–7	8–14	15–29	>30
Cat	1–2	3–8	9–15	>15
Cattle	2–5	6–10	11–20	>20
Horse	Rarely observed	–	–	–
Hypochromasia <sup>†</sup>	1–10	11–50	51–200	>200
Poikilocytosis <sup>†</sup>	3–10	11–50	51–200	>200
Codocytes (Dogs)	3–5	6–15	16–30	>30
Spherocytes <sup>†</sup>	5–10	11–50	51–150	>150
Echinocytes <sup>†</sup>	5–10	11–100	101–250	>250
Other shapes <sup>†</sup>	1–2	3–8	9–20	>20

From Weiss DJ: Uniform evaluation and semiquantitative reporting of hematologic data in veterinary laboratories. Vet Clin Pathol 1984;13:27–31.

\* A monolayer field is defined as a field in which erythrocytes are close together with approximately one half of the erythrocytes touching each other. In severely anemic animals, such monolayers may not be present. When erythrocytes are generally not touching (e.g., tend to be separated by the distance of one cell diameter), then the number of erythrocytes with morphologic abnormalities are counted for two fields.

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- † The same parameters are used for all species, although marked echinocytosis is typically seen in healthy pigs, and marked poikilocytosis is often seen in healthy goats.
- ‡ Parameters are used for acanthocytes, schistocytes, keratocytes, elliptocytes, dacryocytes, drepanocytes, and stomatocytes in all species.

## 3.8.3 Platelet Evaluation

Platelets are small anucleated cells containing magenta-staining granules (Plates 3 to 455), but these granules generally stain poorly in blood obtained from horses. Platelet numbers should be estimated as low, normal, or increased. Blood smears from most domestic animals normally average between 10 and 30 platelets per field when examined with 10× oculars and the 100× objective (1000× magnification). As few as 6 platelets per 1000× field may be present in normal horse blood films. Platelet numbers are generally estimated by multiplying the average number per 1000× field by 20,000 to determine the approximate number of platelets per microliter of blood. Although special attention is given to the estimation of platelet numbers in animals with hemostatic diatheses, it is important to routinely estimate the platelet numbers on blood films, because many animals with thrombocytopenia exhibit no evidence or history of bleeding tendencies. If thrombocytopenia is suspected, it should be confirmed with a platelet count. Dogs and cats have larger platelets than horses and ruminants. The presence of abnormal platelet structure (e.g., large platelets, hypogranular platelets, platelets with inclusions) should be recorded on the hematology form.

## 3.8.4 Infectious Agents and Inclusions

Blood films are examined for the presence of infectious agents and intracellular inclusions by using the 100× objective. Infectious agents and inclusions that may be seen on blood films include Howell-Jolly bodies, Heinz bodies (unstained), basophilic stippling, canine distemper inclusions, siderotic inclusions, Döhle bodies, *Babesia* species, *Cytauxzoon felis*, *Mycoplasma* (*Haemobartonella*) species, *Ehrlichia* species, *Anaplasma* species, *Hepatozoon* species, *Trypanosoma* species, *Theileria* species, *Borrelia* species, and *Bartonella* species. The appearance of these agents and inclusions is presented in subsequent chapters.

## 3.9 ADDITIONAL READING

- MJ Burkhard, G Baxter, MA Thrall: Blood precipitate associated with intra-abdominal carboxymethylcellulose administration. *Vet Clin Pathol.* **25**, 1996, 114–117.
- CB Grindem: Blood cell markers. *Vet Clin North Am Small Anim Pract.* **26**, 1996, 1043–1064.
- J Harenberg, R Malsch, L Piazolo, et al.: Preferential binding of heparin to granulocytes of various species. *Am J Vet Res.* **57**, 1996, 1016–1020.
- JW Harvey: Atlas of veterinary hematology. In *Blood and bone marrow of domestic animals*. 2001, WB Saunders, Philadelphia.
- JS Knoll: Clinical automated hematology systems. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 3–11.
- FW Pierson: Laboratory techniques for avian hematology. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 1145–1147.
- RE Raskin, A Valenciano: Cytochemical tests for diagnosis of leukemia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 755–763.

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RE Raskin, A Valenciano: Cytochemistry of normal leukocytes. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 337–346.

S Tasker, PJ Cripps, AJ Macklin: Estimation of platelet counts on feline blood smears. *Vet Clin Pathol*. **28**, 1999, 42–45.

DJ Weiss: Uniform evaluation and semiquantitative reporting of hematologic data in veterinary laboratories. *Vet Clin Pathol*. **13**, 1984, 27–31.

LL Werner, MM Christopher, J Snipes: Spurious leukocytosis and abnormal WBC histograms associated with Heinz bodies (abstract). *Vet Clin Pathol*. **26**, 1997, 20.

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## 4 Chapter 3 Hematopoiesis and Evaluation of Bone Marrow

### 4.1 OVERVIEW

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#### 4.1.1 Sites of Blood Cell Production

Hematopoiesis begins almost simultaneously outside the body of the embryo in the yolk sac and within the aorta-gonad-mesonephros (AGM) region of the embryo in mammals. Small clusters of hematopoietic stem cells (HSCs) have been identified attached to endothelium in the yolk sac and the dorsal aorta. It is likely that these HSCs and associated endothelial cells are produced by common embryonic stem cells (sometimes referred to as *hemangioblasts*) and that they promote each other's development in a reciprocal manner during embryogenesis. In addition to HSCs, committed erythroid progenitor cells, primitive erythrocytes (large nucleated cells containing embryonal hemoglobin), and rare primitive macrophages are produced in the yolk sacs of rodents and humans. Primitive macrophages appear to develop directly from progenitor cells in the yolk sac without passing through a monocyte stage. Hematopoiesis persists longer during gestation in the yolk sacs of cats than it does in yolk sacs of rodents and humans. In contrast to rodents and humans, in cats definitive erythropoiesis, megakaryocytopoiesis, and limited granulopoiesis also occur in the yolk sac. The AGM region supports the development of HSCs and some committed hematopoietic progenitor cells (HPCs), but recognizable blood cells are not produced in the AGM region.

Sites of blood cell production shift during embryonic and fetal development as optimal microenvironments are produced in various tissues ([Fig. 3-1](#)). The liver and, to a lesser extent, the spleen become the major hematopoietic organs by mid-gestation in the fetus. Current evidence suggests that the AGM region is more important than the yolk sac in providing HSCs to seed the liver and spleen, but the relative importance of each area in embryonic and fetal hematopoiesis remains to be clarified. Blood cell production begins in bone marrow and lymphoid organs during mid-gestation in mammals, with nearly all blood cells being produced in these organs at the time of birth. Blood cells are produced in the bone marrow of adult birds; in the bone marrow and/or spleen of adult reptiles; and in the kidneys, liver, and/or spleen of adult fish and amphibians.

#### 4.1.2 Organization of Bone Marrow

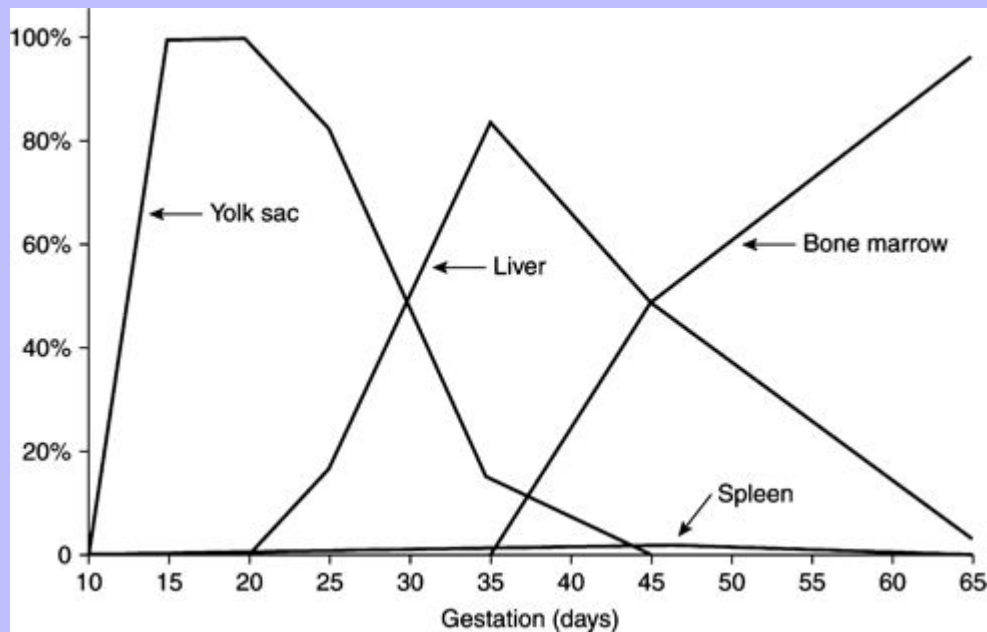
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The bone marrow is a large organ, approximately two thirds the size of the liver in dogs. Bone marrow develops in mammals during the second trimester, after the penetration of cartilage by perichondrial mesenchymal cells and their associated blood vessels, which leads to calcification of the cartilage. The invading vascular mesenchyme forms a connective tissue meshwork, on and within which HSCs originating from the liver (and probably spleen) bind, proliferate, and differentiate, ultimately producing circulating blood cells.

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Fig. 3-1



Sites of definitive hematopoiesis during prenatal development in cats. Percentages represent relative contributions of sites to definitive blood cell production. Lymphoid populations also develop in lymph nodes and thymus beginning in mid-gestation (not shown). (Redrawn from Tiedemann K, van Ooyen B: Prenatal hematopoiesis and blood characteristics of the cat. *Anat Embryol [Berl]* 1978;153:243-267.)

The stroma of the marrow is a connective tissue consisting of stromal cells (also called *reticular cells*), adipocytes, vascular elements (endothelial cells and myocytes), neural elements, and extracellular matrix (ECM). The arrangement of the stroma creates both intravascular and extravascular spaces (Fig. 3-2). Blood cells are continuously produced within the extravascular spaces of bone marrow in postnatal mammals. Leukocytes are also produced within the extravascular spaces of bone marrow in birds, but erythrocytes and thrombocytes are produced within the vascular spaces of the avian marrow.

The specialized arrangement of the marrow vasculature is important in the organization of intramedullary hematopoietic microenvironments. Marrow endothelial cells are actively involved in the regulation of transendothelial (not interendothelial) movement of hematopoietic cells and blood cells between the extravascular hematopoietic space and peripheral blood. Marrow stromal cells have extensively branched cytoplasmic processes. Together with the fibers that they produce, stromal cells provide structural support for the marrow. They have generally been considered to be fibroblast-like, but results of recent studies suggest that stromal cells are vascular smooth muscle-like cells. Stromal cells that support the endothelium of the venous sinuses are termed *adventitial stromal cells*. Granulopoiesis occurs primarily on the surface of stromal cells. Stromal cells and endothelial cells produce the ECM, which consists of collagen fibers, various macromolecules capable of binding cells, and basal laminae of the sinuses. Adipocytes are formed when stromal cells store large quantities of lipid within their cytoplasm. Autonomic nerves of various types are found in bone marrow. Their function is not clear, but is probably largely vasomotor.

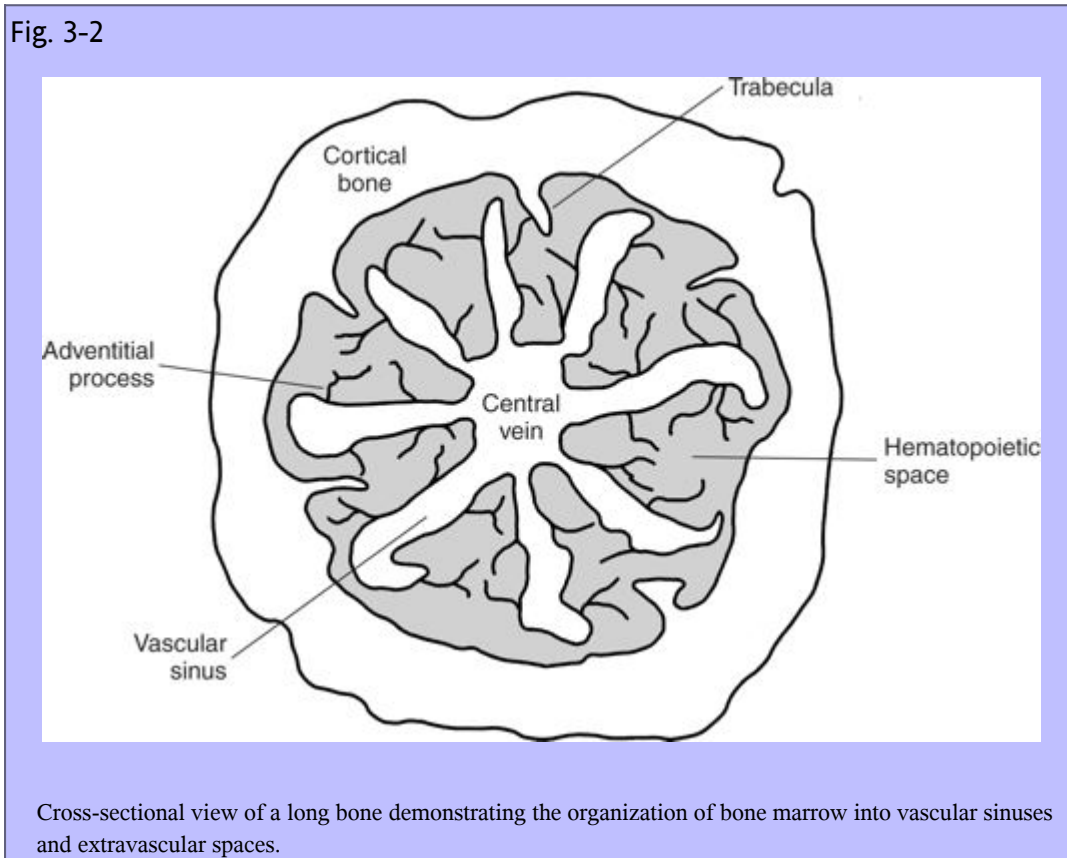
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In addition to hematopoietic cells and developing blood cells, a number of accessory cells involved in regulating hematopoiesis reside within the extravascular space of mammalian bone marrow. These accessory cells include macrophages, mature lymphocytes, and natural killer (NK) cells. Erythrocyte development occurs in close association with marrow macrophages.

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Fig. 3-2



In contrast to other organs, such as skin and intestine, where continuous new cell production occurs throughout life, hematopoietic cells and their progeny in bone marrow are not arranged in stratified layers of progressively more differentiated cells. Although some segregation of cell types may be visualized by microscopic examination of stained bone marrow sections, the overall impression is that bone marrow contains an unstructured mixture of cells of different lineages and stages of maturation. Nonetheless, hematopoietic cells develop in specialized microenvironmental niches within the bone marrow.

### 4.1.3 Hematopoietic Stem Cells and Progenitor Cells

By definition, stem cells are capable of proliferation, continuous self-renewal, and differentiation. The term *hematopoietic progenitor cells (HPCs)* will be used for cells that form colonies in bone marrow culture like HSCs but do not have long-term self-renewal capacities. HSCs and HPCs are mononuclear cells that cannot be distinguished morphologically from lymphocytes. The presence of a transmembrane glycoprotein termed *cluster of differentiation antigen 34 (CD34)* has been used to identify HSCs and early HPCs, but some HSCs

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(presumably inactive ones) lack CD34. In addition, CD34 is also present on the surface of vascular endothelial cells and liver oval cells (presumptive liver stem cells). CD34 is believed to play a role in cell adhesion.

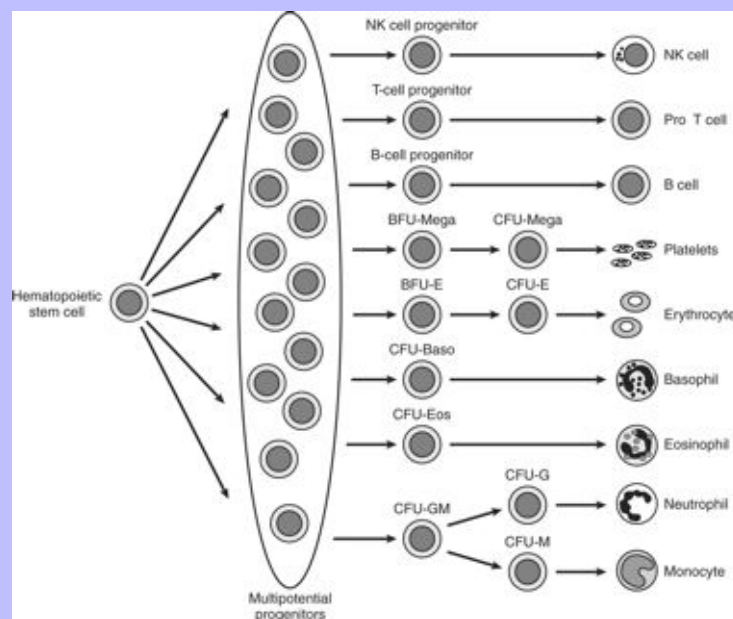
HSCs have the capacity to differentiate into HPCs of all blood cell lineages (erythrocytes, platelets, neutrophils, eosinophils, basophils, monocytes, T lymphocytes, B lymphocytes, and NK cells) and into several cell types in tissue (macrophages, dendritic cells, osteoclasts, and mast cells). The frequency of HSCs in the marrow is estimated to be about 0.01% of nucleated marrow cells in mice and 0.0001% of nucleated marrow cells in cats. Most HSCs are not actively cycling (G0 stage of the cell cycle), but they can be induced to enter the cell cycle and divide by appropriate growth factors. A simplified working model of hematopoietic development is provided (Fig. 3-3). In this model, HSCs produce HPCs that can give rise to two, three, four, or more blood cell types. A common lymphoid progenitor cell is believed to give rise to B lymphocytes (B cells), T lymphocytes (T cells), lymphoid dendritic cells, and NK cells. A common myeloid progenitor cell is believed to give rise to all

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nonlymphoid blood cells, as well as macrophages, myeloid dendritic cells, osteoclasts, and mast cells. HPCs proliferate with higher frequency than do HSCs, but the self-renewal capabilities of HPCs decrease as progressive differentiation and cell lineage restrictions occur. When measured in an in vitro cell culture assay, HPCs are referred to as *colony-forming units (CFUs)*. HPCs that rapidly proliferate, giving rise to multiple subcolonies, are called *burst-forming units (BFUs)*. Colony-forming unit–granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) is a tetrapotential HPC that has been studied extensively in vitro. The existence of a bipotential HPC (colony-forming unit–granulocyte macrophage [CFU-GM]) that is the precursor of both neutrophils and monocytes is well established, and recent studies indicate the likelihood of a bipotential HPC for erythrocytes and megakaryocytes. Although HPCs are portrayed as discrete cell compartments, the transition from one HPC type to another is gradual; consequently, considerable heterogeneity exists within HPC types.

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Fig. 3-3



Simplified working model of hematopoiesis. *NK*, Natural killer; *BFU*, burst-forming unit; *CFU*, colony-forming unit; *Mega*, megakaryocyte; *E*, erythroid; *Baso*, basophil; *Eos*, eosinophil; *G*, granulocyte; *M*, macrophage.

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## 4.1.4 Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) occur in bone marrow at a frequency estimated to be 0.01% to 0.001% of nucleated marrow cells. MSCs have the ability to differentiate into multiple lineages including marrow stromal cells, adipocytes, endothelial cells, osteoblasts, chondrocytes, fibroblasts, and myocytes in smooth, skeletal, and cardiac muscle. Evidence has been provided that suggests that the MSC lineage differs from the HSC lineage in that MSC differentiation pathways are not strictly delineated (i.e., they exhibit plasticity). Apparently, fully differentiated cells of a given lineage may convert into another lineage.

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Stem cells in bone marrow can also give rise to hepatocytes, cholangiocytes, neural cells, and epithelial cells of the skin and gastrointestinal tract. The relationships between these stem cells and HSCs and MSCs need further elucidation, although there is evidence that epithelial cells and neural cells may originate from MSCs.

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## 4.1.5 Homing of Hematopoietic Stem Cells and Progenitor Cells to the Marrow

Homing is the process by which circulating HSCs and HPCs bind to the luminal surface of bone marrow endothelial cells, migrate through the endothelial cells, bind selectively to sites in the extravascular space, and begin the process of proliferation and differentiation. Homing of HSCs and HPCs is mediated by chemoattractants produced by endothelial cells and other cells in the microenvironment and by adhesion molecules expressed on the surfaces of HSCs and HPCs that bind to proteoglycans and glycoproteins on the surfaces of various marrow cells and the ECM.

P-selectin and E-selectin molecules (membrane-spanning, sugar-binding glycoproteins) expressed on bone marrow endothelial cells appear to be involved in initial loose, rolling-type adhesions between HSCs or HPCs and endothelial cells. Tight adhesion and migration through endothelial cells is dependent on integrin molecules, especially  $\beta_1$ -integrins (such as very late antigen-4 or  $\alpha_4\beta_1$ -integrin) on surfaces of migrating cells.

Hematopoietic cells must be activated by locally produced cytokine growth factors for optimal transendothelial migration to occur. These stimulated hematopoietic cells then release cytokines, such as vascular endothelial growth factor (VEGF), which act on endothelial cells, modifying their motility, growth, permeability, and fenestration. The chemokine, stromal cell–derived factor-1 (SDF-1), appears to be especially important in the transendothelial migration of HSCs and HPCs, but other chemoattractants are also involved in this process.

Bone marrow transplantation can be done by injecting bone marrow cells into the blood because of the homing properties of HSCs. In addition, HSCs and HPCs naturally circulate in blood. The mechanisms involved in the release of these hematopoietic cells from bone marrow are not well defined, but HSC and HPC numbers can be increased markedly in blood after injection of growth factors such as granulocyte colony-stimulating factor (G-CSF). In fact, intravenous injection of growth factors is one approach used to collect increased numbers of stem cells from blood for human bone marrow transplantation.

## 4.1.6 Hematopoietic Microenvironment

Blood cell production occurs throughout life in the bone marrow of adult animals because of the unique microenvironment present there. The hematopoietic microenvironment is a complex meshwork composed of stromal cells, endothelial cells, adipocytes, accessory cells (macrophages, subsets of lymphocytes, and NK cells), ECM components, and glycoprotein growth factors that profoundly affect HSC and HPC engraftment, survival, proliferation, and differentiation.

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Stromal cells and endothelial cells produce components of the ECM, including collagen fibers, basement membranes of vessels and vascular sinuses, proteoglycans, and glycoproteins. In addition to providing structural support, the ECM is important in the binding of hematopoietic cells and soluble growth factors to stromal cells and other cells in the microenvironment so that optimal proliferation and differentiation can occur by virtue of these cell-cell interactions (Fig. 3-4).

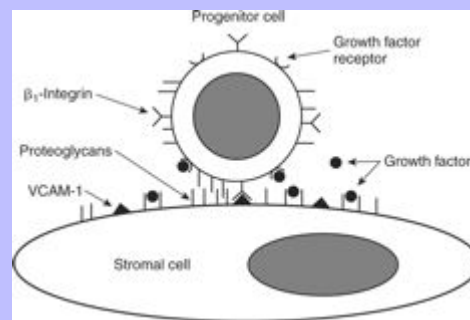
Collagen fibers produced by stromal cells may not have direct stimulatory effects on hematopoiesis, but rather are permissive, promoting hematopoiesis by forming an inert scaffolding around which the other elements of the microenvironment are organized. Hematopoietic cells can adhere to collagen types I and VI.

Adhesion molecules (most importantly  $\beta_1$ -integrins) on the surface of hematopoietic cells bind to ECM glycoproteins such as vascular cell adhesion molecule-1 (VCAM-1), hemonectin, fibronectin, laminin, tenascin, vitronectin, and thrombospondin. The spectrum of expression of adhesion molecules on hematopoietic cells that will differentially bind to these ECM glycoproteins varies with the type, maturity, and activation state of the hematopoietic cells. In addition to anchoring cells to a given microenvironmental niche, binding of adhesion molecules on hematopoietic cells also plays a role in cell regulation by direct activation of signal pathways for cell growth, survival, and differentiation or by modulation of responses to growth factors.

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Fig. 3-4



Interactions between a progenitor cell and stromal cell in the extravascular microenvironment of the bone marrow. *VCAM-1*, Vascular cell adhesion molecule-1.

Proteoglycans are mucopolysaccharides consisting of a protein core with repeating carbohydrate glycosaminoglycans attached. Major proteoglycans in the marrow include heparan sulfate, chondroitin sulfate, hyaluronic acid, and dermatan sulfate. Proteoglycans enhance hematopoiesis by trapping soluble growth factors in the vicinity of hematopoietic cells and by strengthening the binding of hematopoietic cells to the stroma. This is accomplished by proteoglycan binding to molecules, such as platelet endothelial cell adhesion molecule-1 (PECAM-1) and L-selectin, on the surface of hematopoietic cells. Growth factors may also be involved in the adhesion of hematopoietic cells by binding to both the ECM and to specific receptors on hematopoietic cells.

### 4.1.7 Hematopoietic Growth Factors

Proliferation of HSCs and HPCs cannot occur spontaneously, but requires the presence of specific hematopoietic growth factors (HGFs), which may be produced locally in the bone marrow (paracrine or autocrine) or by peripheral tissues and transported to the marrow through the blood (endocrine). All cells in the hematopoietic

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microenvironment, including the hematopoietic cells themselves, produce HGFs, inhibitors of hematopoiesis, or both. Some HGFs have been called *poietins* (erythropoietin [EPO] and thrombopoietin [TPO]). Other growth factors have been classified as colony-stimulating factors (CSFs) on the basis of findings from in vitro culture studies. Finally, some HGFs have been described as interleukins (ILs).

Hematopoietic cells coexpress receptors for more than one HGF on their surfaces. The number of each receptor type present depends on the stage of cell activation and differentiation. Binding of an HGF to its receptor can stimulate proliferation of and alter expression of the HGF receptors on the surface of the cell on which the binding takes place. Some HGFs have permissive hematopoietic effects by inhibiting apoptosis (programmed cell death). The binding of an HGF generally results in down-modulation of its own receptor and up-modulation (transactivation) of receptors for distal HGFs, which primarily act on more differentiated cell types.

HGFs vary in the type(s) of HSCs and HPCs that they can stimulate to proliferate. Factors are often synergistic in their effects on hematopoietic cells. In some instances, an HGF may not directly stimulate the proliferation of a given cell type but may potentiate its proliferation by inducing the expression of membrane receptors for HGFs that do stimulate proliferation. Some glycoproteins such as IL-1 and tumor necrosis factor (TNF) can stimulate hematopoiesis indirectly by stimulating marrow stromal cells, endothelial cells, and T cells to produce HGFs. Different combinations of HGFs regulate the growth of different types of HSCs and HPCs.

Early-acting HGFs are involved in triggering dormant (G0) primitive HSCs to begin cycling. Stem cell factor (SCF) and flt3 ligand (FL) are important early factors that act in combination with one or more other cytokines such as IL-6, IL-11, IL-12, G-CSF, and leukemia inhibitory factor. In addition to promoting the growth of HSCs, SCF and FL appear to promote the growth of granulocytic and monocytic progenitor cells, but only SCF appears to be important in promoting the growth of erythroid and megakaryocytic progenitor cells.

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Intermediate-acting HGFs have broad specificity. IL-3 (multi-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-4 support proliferation of multipotent HPCs. These factors also interact with late-acting factors to stimulate proliferation of a wide variety of committed progenitor cells. Late-acting HGFs have restricted specificity. G-CSF, macrophage colony-stimulating factor (M-CSF), EPO, TPO, and IL-5 are more restrictive in their actions. They have their most potent effects on committed progenitor cells and later stages of development when cell lines can be recognized morphologically.

## 4.2 ERYTHROPOIESIS

### 4.2.1 Primitive Erythropoiesis

Primitive erythrocytes are large (about 500 fL in mice) nucleated cells with high nuclear to cytoplasmic ratios. Their nuclei have open (noncondensed) chromatin, and their cytoplasm contains predominantly embryonal hemoglobin. These primitive erythrocytes do not depend on EPO. Primitive erythrocyte production begins in the yolk sac but also occurs later in the liver and bone marrow. A switch to definitive erythropoiesis occurs during fetal development. Definitive erythropoiesis results in the production of smaller cells that extrude their nuclei, produce fetal hemoglobin (in some species) and adult hemoglobin, and are highly dependent on EPO.

### 4.2.2 Hematopoietic Progenitor Cells and the Bone Marrow Microenvironment

Oligopotent progenitor cells (including CFU-GEMM cells) are stimulated to proliferate and differentiate into burst-forming unit–erythroid (BFU-E) cells by SCF, IL-3, and GM-CSF in the presence of EPO. BFU-E cell proliferation and differentiation into colony-forming unit–erythroid (CFU-E) cells results from the presence of

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these same factors and may be further potentiated by additional growth factors. EPO is the primary growth factor involved in the proliferation and differentiation of CFU-E cells into rubriblasts, the first morphologically recognizable erythroid cells. CFU-E cells are more responsive to EPO than are BFU-E cells because CFU-E cells exhibit greater numbers of surface receptors for EPO.

Marrow macrophages appear to be an important component of the hematopoietic microenvironment involved with erythropoiesis. Both early and late stages of erythroid development occur with intimate membrane apposition to central macrophages in so-called erythroblastic islands. These central macrophages may regulate basal erythrocyte production by producing both positive growth factors, including EPO, and negative factors such as IL-1, TNF, and interferons (IFNs). The finding that EPO can also be produced by erythroid progenitors suggests that these cells may support erythropoiesis by autocrine stimulation. Although some degree of basal regulation of erythropoiesis occurs within the marrow microenvironment, humoral regulation is also important, with EPO production occurring primarily within peritubular interstitial cells of the kidneys and various inhibitory cytokines being produced at sites of inflammation throughout the body.

### 4.2.3 Nutrients Needed for Erythropoiesis

In addition to amino acids and essential fatty acids, several metals and vitamins are required for normal erythropoiesis. Iron is needed for the synthesis of heme, an essential component of hemoglobin and certain enzymes. Copper, in the form of ceruloplasmin, is important in the release of iron from tissue to plasma for transport to developing erythroid cells. Vitamin B<sub>6</sub> (pyridoxine) is needed as a cofactor in the first enzymatic step in heme synthesis.

Tetrahydrofolic acid, the active form of folic acid (a B vitamin), is needed for the transfer of single carbon-containing molecules in DNA and RNA synthesis. The physiologic mechanism of vitamin B<sub>12</sub> involvement in erythrocyte production is not well understood, but it is interrelated with folate metabolism. Cobalt is essential for the synthesis of vitamin B<sub>12</sub> by ruminants.

### 4.2.4 Maturation of Erythroid Cells

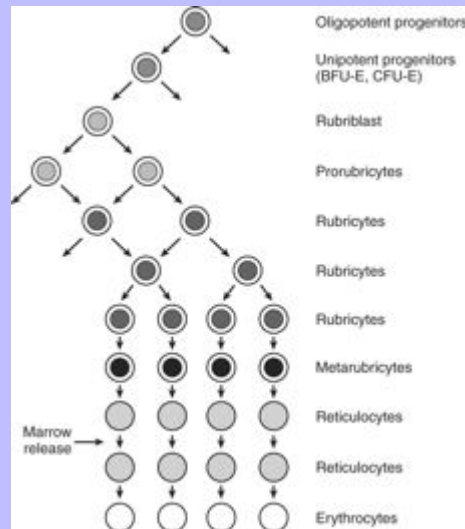
Rubriblasts are continuously generated from progenitor cells in the extravascular space of the bone marrow. The production of a rubriblast initiates a series of approximately four divisions over a period of 3 or 4 days to produce about 16 metarubricytes that are no longer capable of division (Fig. 3-5). These divisions are called *maturational divisions* because there is a progressive maturation of the nucleus and cytoplasm concomitant with each division.

Early precursors have intensely blue cytoplasm, when stained with Romanowsky-type blood stains, owing to the presence of many basophilic ribosomes and polyribosomes that are actively synthesizing globin chains and smaller amounts of other proteins. As these cells divide and mature, overall cell size decreases, nuclear chromatin condensation increases, cytoplasmic basophilia decreases, and hemoglobin progressively accumulates, imparting a red coloration to the cytoplasm (Plate 19). Cells with both red and blue coloration are described as having polychromatophilic cytoplasm. An immature erythrocyte, termed a *reticulocyte*, is formed after extrusion of the metarubricyte nucleus. Extruded nuclei are bound and phagocytosed by a novel receptor on the surface of bone marrow macrophages.

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Fig. 3-5



A diagram of erythropoiesis showing the release of reticulocytes into blood as it normally occurs in dogs. *BFU-E*, Burst-forming unit–erythroid; *CFU-E*, colony-forming unit–erythroid.

Early reticulocytes have polylobulated surfaces. Their cytoplasm contains ribosomes, polyribosomes, and mitochondria necessary for the completion of hemoglobin synthesis. Reticulocytes derive their name from a network, or reticulum, that appears when blood is stained with basic dyes such as new methylene blue and brilliant cresyl green. That network is not preexisting but is an artifact formed by the precipitation of ribosomal ribonucleic acids and proteins as a result of staining. As reticulocytes mature, the amount of ribosomal material decreases until only a few basophilic specks can be visualized with reticulocyte staining procedures. These mature reticulocytes have been referred to as *punctate reticulocytes*.

Reticulocyte maturation into mature erythrocytes is a gradual process that requires a variable number of days, depending on the species involved. Consequently, the morphologic and physiologic properties of reticulocytes vary with the stage of maturation. The cell surface undergoes extensive remodeling with the selective loss of certain membrane protein and lipid components, and ultimately, the formation of the biconcave shape of mature erythrocytes. Mitochondria and ribosomes are also lost during maturation by energy-dependent mechanisms.

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Reticulocyte maturation begins in the bone marrow and is completed in the peripheral blood and spleen in dogs, cats, and pigs. As reticulocytes mature, they lose the surface receptors needed to adhere to fibronectin and thrombospondin within the ECM, thereby facilitating their release from the bone marrow.

Reticulocytes become progressively more deformable as they mature, a characteristic that also facilitates their release from the marrow. To exit the extravascular space of the marrow, reticulocytes press against the abluminal surfaces of endothelial cells that make up the vascular sinus wall. Endothelial cell cytoplasm thins and small pores develop, allowing reticulocytes to be pushed through by a small pressure gradient across the sinus wall. The pores apparently close after reticulocyte passage.

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Relatively immature, aggregate-type reticulocytes are released from canine bone marrow; consequently, most of these cells appear polychromatophilic when viewed following routine blood film staining procedures. Reticulocytes are usually not released from the bone marrow of nonanemic cats until they mature to punctate-type reticulocytes (Fig. 3-6); consequently, few or no aggregate reticulocytes (less than 0.4%), but up to 10% punctate reticulocytes, are found in blood from healthy adult cats. The high percentage of punctate reticulocytes results from a long maturation time with delayed degradation of organelles. Reticulocytes are generally absent in peripheral blood of healthy adult cattle and goats, but a small number of punctate types (0.5%) may be found in the blood of adult sheep. Equine reticulocytes are absent from blood normally and are not released in response to anemia.

4.2.5

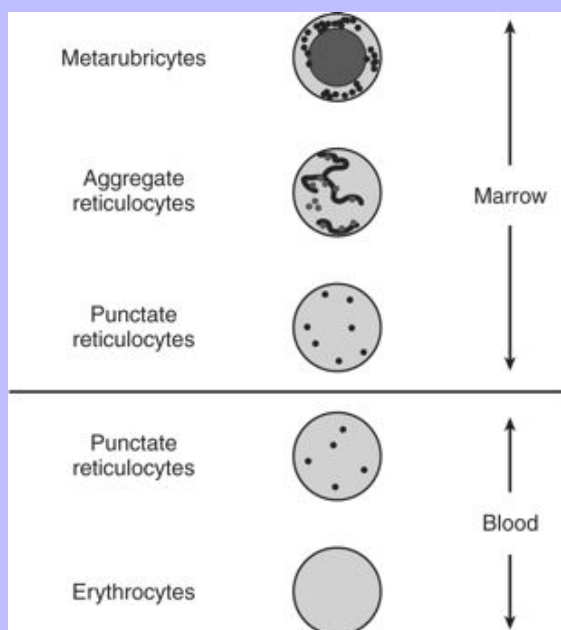
### Control of Erythropoiesis

EPO is a 30- to 34-kd glycoprotein. Its production is stimulated by tissue hypoxia in the kidneys (Fig. 3-7). The oxygen sensor has not been defined, but there is evidence that a heme protein may be involved. Tissue oxygen tension is determined by the oxygen consumption of the tissue and the oxygen-delivering capacity of the blood. Oxygen-delivering capacity depends on cardiovascular integrity, oxygen content in arterial blood, and hemoglobin oxygen affinity. Low oxygen content in the blood can result from low partial pressure of oxygen ( $PO_2$ ) in arterial blood, as occurs with high altitudes or with congenital heart defects in which some of the blood flow bypasses the pulmonary circulation. Low oxygen content in blood can also occur with normal  $PO_2$ , as occurs with anemia and methemoglobinemia. An increased oxygen affinity of hemoglobin within erythrocytes results in a decreased tendency to release oxygen to the tissues.

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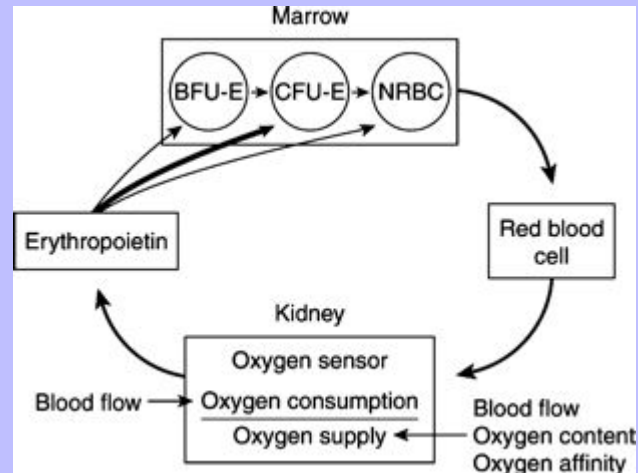
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Fig. 3-6



Drawing of cat erythroid cells (stained with the reticulocyte stain) demonstrating reticulocyte release into blood as it occurs in most healthy cats.

Fig. 3-7

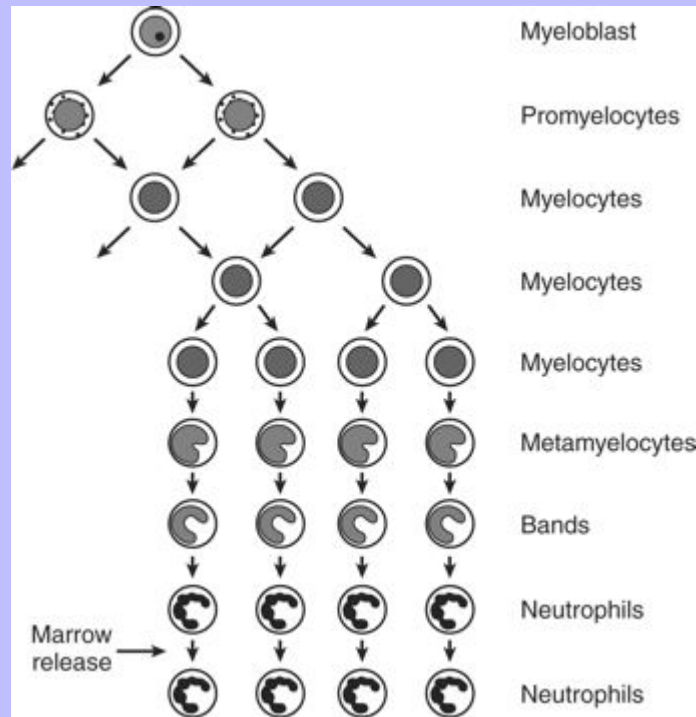


Central role of erythropoietin (EPO) in the control of erythropoiesis. *BFU-E*, Burst-forming unit–erythroid; *CFU-E*, colony-forming unit–erythroid; *NRBC*, nucleated red blood cell.

High plasma concentrations of EPO may accelerate rubriblast entry into the first mitotic division, shortening the marrow transit time and resulting in the early release of basophilic macroreticulocytes called *stress reticulocytes* or *shift reticulocytes*. Although EPO has been assumed to act as a mitogen because of its capacity to amplify erythrocyte production, some studies have suggested that it acts primarily as a survival factor, preventing apoptosis and permitting cells to proceed with programmed proliferation and maturation. In the presence of EPO, other hormones—including androgens, thyroid hormones, and growth hormone—can enhance the growth of erythroid precursor cells.

Hematopoietic cells die not only as a consequence of lack of HGFs but also in response to the presence of molecules that induce apoptosis. Inhibitors of erythropoiesis include transforming growth factor- $\beta$  (TGF- $\beta$ ), TNF- $\alpha$ , IFN- $\gamma$ , Fas ligand, and TNF-related apoptosis-inducing ligand (TRAIL).

Fig. 3-8



Granulopoiesis.

## 4.3 LEUKOPOIESIS

### 4.3.1 Neutrophil Production

Neutrophilic cells within the bone marrow can be included in two pools (Fig. 3-8). The proliferation and maturation pool (mitotic pool) includes myeloblasts, promyelocytes, and myelocytes. Approximately four or five divisions occur over several days. During this time, primary (reddish purple) cytoplasmic granules are produced in late myeloblasts or early promyelocytes, and secondary (specific) granules are synthesized within myelocytes. Once nuclear indentation and condensation become apparent, precursor cells are no longer capable of division. The maturation and storage pool (postmitotic pool) includes metamyelocytes, bands, and segmented neutrophils. Cells within this pool normally undergo maturation and storage for several more days before the migration of mature neutrophils through the vascular endothelium and into the circulation. The marrow transit time from myeloblast formation to release of mature neutrophils into blood varies by species but is generally between 6 and 9 days. This time can be shortened considerably when inflammation is present.

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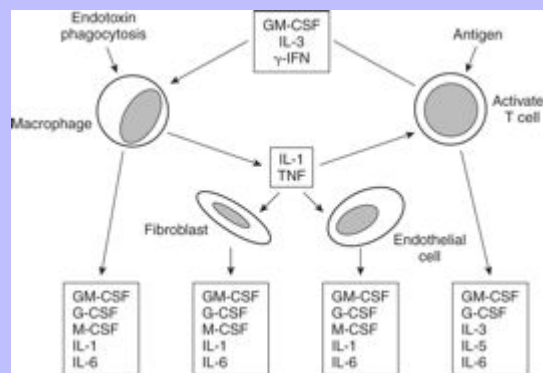
The normal release of mature neutrophils from the marrow involves the maturational loss of receptors for ECM proteins such as hemonectin. The number of mature neutrophils stored in marrow is several times the number present in the circulation.

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A variety of cytokines with overlapping specificities are important in neutrophil production. Oligopotent cells (e.g., CFU-GEMM) are stimulated to proliferate (and possibly differentiate) by SCF, FL, and IL-3. One progeny produced is a bipotential progenitor cell type (CFU-GM), which is the precursor of both neutrophils and monocytes (see Fig. 3-3). CFU-GM cells are stimulated to proliferate and differentiate by GM-CSF. At low concentrations, GM-CSF stimulation favors the formation of mononuclear phagocytes, and at high concentrations, the formation of neutrophils. Unipotential colony-forming unit-granulocyte (CFU-G) cells are stimulated to proliferate and differentiate into myeloblasts by G-CSF. This cytokine appears to play a role in the basal regulation of granulopoiesis, as well as function as a primary regulator of the neutrophil response to inflammatory stimuli. G-CSF increases the number of cell divisions and reduces the time for maturing neutrophil precursors to develop into terminally differentiated cells and be released into blood.

Fig. 3-9



Activation of T cells and macrophages stimulates the production of cytokines that directly or indirectly stimulate hematopoiesis. *GM-CSF*, Granulocyte-macrophage colony-stimulating factor; *IL*, interleukin; *γ-IFN*,  $\gamma$ -interferon; *TNF*, tumor necrosis factor; *G-CSF*, granulocyte colony-stimulating factor; *M-CSF*, macrophage colony-stimulating factor.

Activated helper T cells produce various cytokines including IL-3 and GM-CSF. Mononuclear phagocytes, fibroblasts, and endothelial cells can produce GM-CSF and G-CSF when appropriately stimulated. Not only can mononuclear phagocytes synthesize HGFs when they contact bacterial products, they can also stimulate other cells to produce them. The monokines IL-1 and TNF stimulate the production of HGFs by other cell types (Fig. 3-9). These monokines are important in the inflammatory response to foreign organisms and neoplastic cells but may not be involved in resting granulopoiesis.

Inhibition of neutrophil production is not well understood, but mature neutrophils within the marrow inhibit neutrophilic colony formation. Serine proteases, such as elastase released from neutrophils, can inhibit granulopoiesis. When appropriately stimulated, neutrophils also secrete IFN- $\alpha$ , which inhibits neutrophil production. Increased neutrophil numbers in blood are associated with increased clearance of circulating G-CSF after binding to surface receptors on neutrophils, thereby producing a negative feedback on granulopoiesis. Mature neutrophils also indirectly inhibit granulopoiesis by removal (phagocytosis) of invading microorganisms that would otherwise result in the production of HGFs by tissue cells.

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## 4.3.2 Eosinophil, Basophil, and Mast Cell Production

Eosinophil production in marrow parallels that of neutrophils. The marrow transit time is 1 week or less, with a significant storage pool of mature eosinophils. As for neutrophils, growth factors such as IL-3 and GM-CSF are needed for proliferation of early progenitors. Activated T cells produce IL-5, which promotes the terminal maturation of eosinophils. Inhibitors of eosinophil production include IL-12 and IFN- $\gamma$ .

Basophils and eosinophils appear to share a common marrow progenitor cell that gives rise to precursors specific for each lineage. Eosinophil and basophil precursors become recognizable at the myelocyte stage when their characteristic secondary granules appear. SCF, IL-3, IL-4, IL-5, and probably other cytokines stimulate basophil production. It is unclear whether basophils and mast cells share a common progenitor cell. In contrast to maturation of basophils, which occurs in the bone marrow, maturation of mast cell progenitors into mast cells occurs in the tissues. SCF, but not FL, is essential for mast cell production. Some local proliferation of mast cells can occur in tissues if mast cells are appropriately stimulated.

## 4.3.3 Monocyte Production

Monocytes are produced through the combined effects of IL-3, GM-CSF, and M-CSF on proliferation and differentiation of bone marrow progenitor cells. Less time is required to produce monocytes than granulocytes, and there is little marrow reserve of monocytes. Monocytes are not end-stage (finished) cells but must enter the tissues to become macrophages. M-CSF acts on later progenitor cells than the other two CSFs and induces predominantly macrophages.

Monocyte progenitors or monocytes themselves are also capable of producing myeloid dendritic cells and osteoclasts. Mature myeloid dendritic cells are produced in culture by the combined effects of GM-CSF, IL-4, and TNF- $\alpha$ . M-CSF inhibits myeloid dendritic cell formation. IFN- $\gamma$  promotes macrophage development in preference to myeloid dendritic cell production.

Osteoclasts develop when monocyte progenitors are cultured with M-CSF and a soluble form of receptor activator of nuclear factor- $\kappa$ B ligand (RANKL). IL-3 and GM-CSF inhibit osteoclast formation. The relative amounts of these growth factors, and presumably others, present in the microenvironment of a monocyte progenitor apparently determine whether macrophages, myeloid dendritic cells, or osteoclasts are formed.

## 4.3.4 Lymphocyte, Natural Killer Cell, and Dendritic Cell Production

HSCs of the bone marrow give rise to a common lymphoid progenitor cell, which can produce B-cell progenitors, T-cell progenitors, NK cell progenitors, and lymphoid dendritic cell progenitors. The development of B-cell and T-cell progenitors in bone marrow is antigen-independent. Both SCF and FL appear to be involved in the production of early lymphoid progenitor cells.

B-cell progenitors produce mature, naive B cells in the marrow in most mammals, in ileocecal Peyer's patches in ruminants, and in the bursa of Fabricius in birds. As with other blood cells, the microenvironment of the marrow and lymphoid organs is important for lymphopoiesis. The production of antigen-sensitive, surface immunoglobulin-positive B cells is marked by successive rearrangements of the immunoglobulin gene loci and selective expression of surface proteins. Although a number of cytokines including SCF, FL, IL-3, and insulin-like growth factor are involved in B-cell production in marrow, IL-7 appears to be an especially important positive growth factor. B-cell lymphopoiesis is inhibited by several factors including TGF- $\beta$ , IFN- $\gamma$ ,

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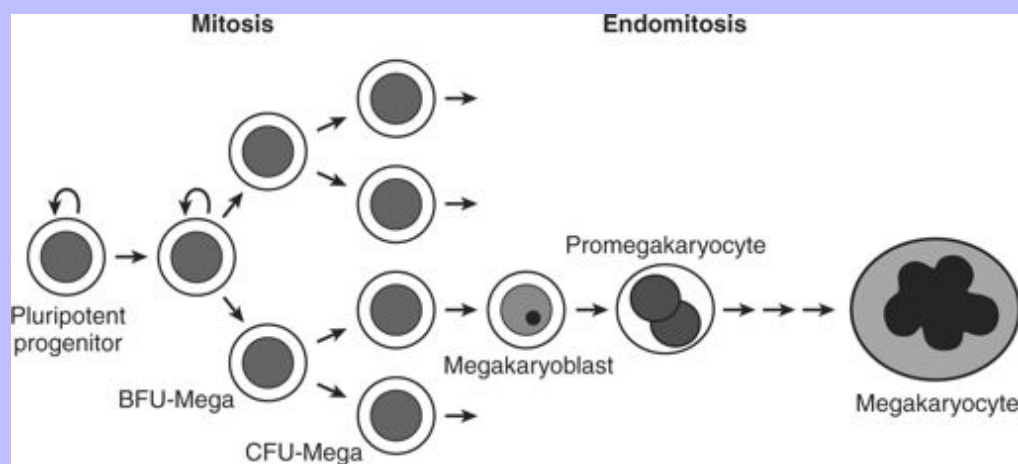
IFN- $\alpha$ , and limitin, an IFN-like cytokine. Recirculating B cells are activated by antigenic stimulation in the T-cell region of secondary lymphoid organs, followed by migration to the cortex in lymph nodes and to follicles in jejunal Peyer's patches and spleen in mammals. B cells activated by antigen in close contact with dendritic cells can differentiate into plasmablasts that can develop into plasma cells in lymphoid organs where they are produced, or plasmablasts can migrate through blood and develop into plasma cells in bone marrow. IL-6 appears to promote plasma cell development by preventing apoptosis of plasmablasts.

T-cell progenitors leave the marrow and migrate to the thymus. Homing of these cells to the thymus depends on their interaction with various adhesion molecules on thymic/endothelial cells and the production of specific chemotactic factors by thymic stromal cells. T-cell progenitors develop into T cells under the influence of the thymic microenvironment and growth factors (including SCF and IL-7) produced in the thymus. T-cell lymphopoiesis is inhibited by several factors including IFN- $\gamma$  and IFN- $\alpha$ . After maturation in the thymus, T cells accumulate within paracortical areas of lymph nodes, periarteriolar lymphoid sheaths of the spleen, and the interfollicular areas of jejunal Peyer's patches in mammals.

NK cells are primarily produced and undergo maturation in the bone marrow, but NK progenitor cells are also present in the thymus. Growth factors controlling their production have not been well characterized; but SCF, FL, IL-2, IL-7, and IL-15 can stimulate NK cell development from progenitor cells. IL-4 inhibits NK cell colony formation in culture. NK cells are located primarily in blood and the spleen, with low numbers in lymph nodes in healthy mammals. The mucosal lymphocytes (interepithelial lymphocytes of the small intestine) may represent a subset of NK cells.

Dendritic cells can develop from common myeloid progenitor cells and common lymphoid progenitor cells. Lymphoid dendritic cells appear to share a later progenitor cell with T cells and NK cells, but not with B cells. Myeloid dendritic cells appear to share a later progenitor cell with granulocytes and macrophages, but not with erythrocytes and megakaryocytes. Lymphoid dendritic cells can form in the bone marrow or thymus, with IL-7 being important in the production of these cells.

Fig. 3-10



Stages of megakaryocyte development. *BFU-Mega*, Burst-forming unit-megakaryocyte; *CFU-Mega*, colony-forming unit-megakaryocyte.

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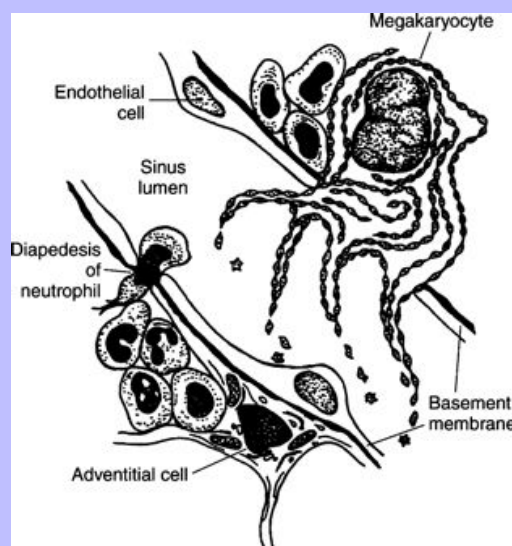
## 4.4 THROMBOPOIESIS

Blood platelets in mammals are produced from multinucleated giant cells in bone marrow called *megakaryocytes*. The earliest definable progenitor cell committed to form megakaryocytes is termed *BFU-megakaryocyte* (*BFU-Mega*). When appropriately stimulated, this progenitor cell divides and differentiates into CFU-Mega progenitor cells, which divide and differentiate into megakaryoblasts (Fig. 3-10). Mitosis stops at this stage and endomitosis (nuclear reduplication without cell division) begins. Generally, two to five nuclear reduplications occur, resulting in 8 to 64 sets of chromosomes (8N-64N) in mature megakaryocytes, compared with two sets of chromosomes (2N) in most cells in the body. Individual nuclei can be observed after the first two reduplications (promegakaryocytes), but a large polylobulated nucleus is seen when mature megakaryocytes are formed. The mean ploidy of human megakaryocytes (16N) is lower than mean values (32N to 64N) reported for megakaryocytes in dogs, cats, and cattle. The cytoplasm in promegakaryocytes is intensely basophilic. A progressive decrease in basophilia and an increase in granularity occur as megakaryocytes mature. Cell volume increases with each reduplication; consequently, megakaryocytes are much larger than all other marrow cells except osteoclasts. In contrast to mature megakaryocytes, osteoclasts have multiple discrete nuclei.

Megakaryocytes either lie just outside a vascular sinus or compose part of the wall of a sinus. Cylinders of cytoplasm from megakaryocytes form and extend into sinuses (Fig. 3-11). These beaded-appearing proplatelets eventually fragment into individual platelets within the sinuses and general circulation. Megakaryocytes may also migrate through the vascular endothelium into the sinuses, enter the general venous circulation, and become lodged in pulmonary capillaries where platelet formation can also occur. The chemokine stromal cell–derived factor 1 (SDF-1) appears to be important in promoting this migration through the sinus endothelium. It is estimated that 1000 to 5000 platelets are produced from each megakaryocyte, depending on megakaryocyte size. Megakaryocytes are not present in nonmammalian species. Like erythrocytes and leukocytes, the nucleated thrombocytes of nonmammalian species are produced by mitosis of precursor cells.

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Fig. 3-11



Extension of proplatelets into the sinus lumen and the subsequent formation of individual platelets.

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A number of cytokines can stimulate or enhance the proliferation and expansion of megakaryocyte progenitor cells. Factors that may be involved include SCF, FL, IL-3, and GM-CSF. TPO increases platelet production by stimulating megakaryocyte differentiation from marrow progenitor cells and by stimulating increased endomitosis, resulting in increased ploidy and megakaryocyte size. SDF-1 appears to work in concert with TPO in promoting the formation of large proplatelet-bearing polyploid megakaryocytes. TPO may also transiently enhance the aggregatory response of platelets to agonists.

Although various cells in the body can produce TPO, including cells within the bone marrow microenvironment, the major sites of TPO production appear to be the parenchymal and sinusoidal endothelial cells of the liver and proximal convoluted tubules of the kidneys. The amount of TPO produced in the body appears to be relatively constant. TPO receptors (c-Mpl) on blood platelets and maturing megakaryocytes can bind, internalize, and degrade TPO, providing negative feedback on platelet production. Consequently, the blood TPO concentration is remarkably high when thrombocytopenia resulting from megakaryocytic hypoplasia is present. In contrast, blood TPO concentrations are much lower in animals with ongoing immune-mediated thrombocytopenia, because megakaryocytes are generally increased in the marrow and rapid platelet turnover is occurring, resulting in increased binding and removal of TPO from blood. However, the number of maturing megakaryocytes and blood platelets present may not be the only determinants of blood TPO concentrations. Increased platelet production occurs after administration of IL-6, and this inflammatory cytokine appears to stimulate increased TPO synthesis. TGF- $\beta$  and TNF- $\alpha$  appear to be inhibitors of megakaryocyte production.

## 4.5 BONE MARROW BIOPSY AND EVALUATION

### 4.5.1 Reasons for Bone Marrow Examination

Bone marrow evaluation is indicated when peripheral blood abnormalities are detected. The most common indications are persistent neutropenia, unexplained thrombocytopenia, poorly regenerative anemia, or a combination thereof. Examples of proliferative abnormalities in which bone marrow examination may be indicated include persistent thrombocytosis or leukocytosis, abnormal blood cell morphology, or the unexplained presence of immature cells in blood (e.g., nucleated erythroid cells in the absence of polychromasia or a neutrophilic left shift in the absence of inflammation).

Bone marrow is sometimes examined to stage neoplastic conditions (lymphomas and mast cell tumors); estimate the adequacy of body iron stores; evaluate lytic bone lesions; and search for occult disease in animals with fever of unknown origin, unexplained weight loss, and unexplained malaise. Bone marrow examination can also be useful in determining the cause of a hyperproteinemia when it occurs in association with multiple myeloma, lymphoma, leishmaniasis, or systemic fungal diseases. It may also reveal the cause of hypercalcemia when it is associated with lymphoid neoplasms, multiple myeloma, or metastatic neoplasms to bone.

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Bone marrow aspirate biopsies are done more frequently than core biopsies in veterinary medicine. Aspirate biopsies are easier, faster, and less expensive to perform than are core biopsies. Bone marrow core biopsies require special needles that cut a solid core of material, which is then placed in fixative, decalcified, embedded, sectioned, stained, and examined microscopically by a pathologist. Core biopsy sections provide a more accurate means of evaluating marrow cellularity and examining tissue for metastatic neoplasia than do aspirate smears, but cell morphology is more difficult to assess.

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## 4.5.2 Bone Marrow Aspiration Technique

There are few contraindications to a bone marrow aspiration biopsy. Restraint, sedation, and anesthesia (when used) generally pose more risks to the patient than the biopsy procedure itself. Postbiopsy hemorrhage is a potential complication in patients with hemostatic diatheses, but it rarely occurs. Hemorrhage may occur after biopsy is performed in animals with monoclonal hyperglobulinemia, but it is easily controlled by placing a suture in the skin incision and applying pressure over the biopsy site. Postbiopsy infection is also a potential but highly unlikely complication if proper techniques are used.

The usefulness of bone marrow aspirate cytology as a diagnostic aid depends on the proper collection of the bone marrow sample and preparation of high-quality marrow smears. In most cases only local anesthesia is needed for needle biopsies. Tranquilization is sometimes used in patients that resist positioning by manual restraint. Biopsy sites are prepared by clipping the hair and scrubbing the skin with antiseptic soap preparations. A local anesthetic is injected under the skin and down to the periosteum overlying the site from which the biopsy specimen is to be obtained, and a small skin incision is made with a scalpel blade to facilitate passing the needle through the skin. Sterile needles and gloves are always used. If general anesthesia is required for other procedures, bone marrow aspiration may be scheduled at the same time to minimize the stress on the animal.

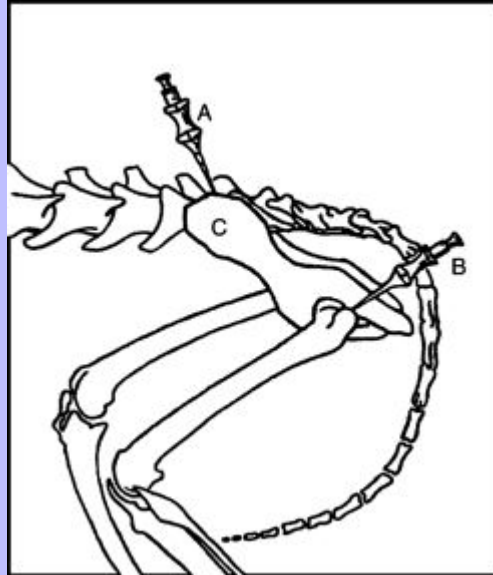
The biopsy needle used to aspirate marrow must have a removable stylet that remains in place until the marrow cavity is entered to prevent obstruction of the needle lumen with cortical bone. A 16- or 18-gauge Rosenthal needle that is between 1 and 1.5 inches long is satisfactory. The wing of the ilium is often used as a site from which to obtain marrow in dogs and large cats, unless biopsy specimens of specific lesions are to be obtained on the basis of radiographic findings (Fig. 3-12). For small cats and toy breeds of dogs in which the ilium is especially thin, one may aspirate from the head of the proximal femur by way of the trochanteric fossa.

Aspiration of marrow from the anterior side of the proximal end of the humerus is another popular method, especially in obese patients. In horses and large dogs, the third, fourth, or fifth sternebra can be used as a biopsy site. Sternebra biopsies have the risk of inadvertent penetration of the thorax and damage to structures in the thoracic cavity. A short biopsy needle (preferably with an adjustable guard) should be used, and care should be taken to keep the needle in the center of these bones to minimize the risk of pneumothorax, uncontrolled hemorrhage, or cardiac laceration.

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Fig. 3-12



Bone marrow biopsy methods include (A) positioning the needle in the widened dorsal aspects of the iliac crest, (B) positioning the needle in the trochanteric fossa of the proximal femur, and (C) positioning the needle in the central depression in the wing of the ileum. (From Harvey JW: Canine bone marrow: Normal hematopoiesis, biopsy techniques, and cell identification and evaluation. *Comp Contin Educ Pract Vet* 1984;6:909.)

For the purpose of entering the marrow space, moderate pressure is applied to the needle (with the stylet locked in place) as the needle is rotated in an alternating clockwise-counterclockwise motion. Once the needle is firmly embedded in the bone, it is usually within the marrow cavity. The stylet is then removed, and a 10-mL or 20-mL syringe is attached to the needle. Vigorous negative pressure should be applied by rapidly pulling the plunger back as far as possible. As soon as a few drops of blood appear in the syringe, the negative pressure is released and the complete assembly is rapidly removed for smear preparation. If marrow does not appear in the syringe, the stylet is replaced, and the needle is repositioned for another aspiration attempt.

For accurate bone marrow evaluation, smears must contain marrow particles (stroma and associated cells). Marrow particles appear as small white grains. One useful method that assists in the separation of particles from contaminating blood involves the forcible expulsion of several drops of the marrow aspirate onto one end of a glass slide, which is then held vertically (Fig. 3-13). Particles tend to stick to the slide, whereas blood runs off. A second glass slide is placed across the area of particle adherence perpendicular to the first slide, and after marrow spreads between the slides, they are pulled apart in the horizontal plane. Resultant smears are rapidly air-dried. Since marrow clots rapidly, one may collect marrow into a syringe that contains several drops of 4% ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Marrow is mixed with the anticoagulant and expelled into a weighing dish or Petri dish. Particles are then collected by capillary tube or pipette, and squash preparations are made.

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Smears are stained with a Romanowsky-type blood stain such as Wright, Giemsa, or a combination thereof. Satisfactory results can usually be obtained with the Diff-Quik stain, a rapid modified Wright stain. If adequate smears are available, one smear should be stained by using the Prussian blue procedure for iron. Additional special stains may be required to help differentiate the type of leukemia when it is detected.

## 4.5.3 Bone Marrow Aspirate Cytology

Bone marrow should be examined and findings recorded in a systematic manner. Smears should be scanned with low-power objectives to gain an appreciation of overall cellularity and determine the adequacy of megakaryocyte numbers. Normal marrow appears heterogenous. As a rule, erythroid precursors are smaller, have more nearly spherical nuclei with more condensed nuclear chromatin, and have darker cytoplasm than do granulocyte precursors at similar maturation stages (Plate 19). Rubriblasts have deeper basophilic cytoplasm than myeloblasts, promyelocytes, or myelocytes. Consequently, smaller and darker cells, observed by scanning marrow smears at low power, are usually erythroid precursors (unless lymphocytes are increased in number) and the larger, paler cells are usually granulocyte precursors.

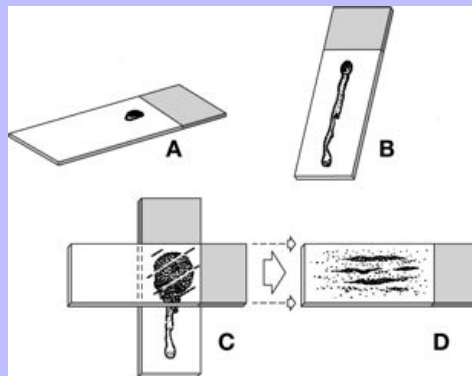
Complete differential cell counts are not usually done on marrow smears; rather, a number of judgments are made and recorded as follows.

1. The cellularity of the marrow is estimated by examining the proportion of cells versus fat present in particles. Marrow particles appear as blue-staining areas on gross examination. Microscopic examination reveals that they contain vessels, stromal cells, macrophages, plasma cells, and blood cell precursors (Fig. 3-14). Most particles in healthy animals are composed of one third to two thirds cells. If few or no particles are present on smears, it is not possible to accurately estimate marrow cellularity. 42  
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2. The frequency and morphology of megakaryocytes should be evaluated. Most large particles should have associated megakaryocytes, and normally, a majority of megakaryocytes are of the granular, mature type. Any abnormal megakaryocyte morphology (e.g., dwarf megakaryocytes) should be noted.
3. The distribution of granulocytic cells should be evaluated to determine whether the series is complete (i.e., a normal number of mature granulocytes are present) and orderly. If there are increased proportions of cells in the proliferating pool (myeloblasts, promyelocytes, or myelocytes) compared with the later stages of development, this finding should be noted. Morphologic abnormalities, such as vacuolated cytoplasm, or increased representation of eosinophilic or basophilic series should be noted.
4. The maturation and morphology of the erythroid series should be evaluated to determine whether it is complete (frequent polychromatophilic erythrocytes should be present) and orderly. Possible abnormal morphologic findings that should be noted include megaloblastic cells, frequent binucleate cells, and pleomorphic nuclei.
5. A myeloid to erythroid (M/E) ratio is calculated by examining 500 cells and determining the ratio of granulocytic cells (including mature granulocytes) to nucleated erythroid cells. The M/E ratio is generally between 0.75 and 2.5 in healthy dogs, between 1.2 and 2.2 in healthy cats, and between 0.5 and 1.5 in healthy horses. One needs to have a knowledge of the overall marrow cellularity, as well as the complete blood count (CBC), to interpret the M/E ratio. Examples of dog bone marrow aspirate smears with high (Fig. 3-15) and low (Fig. 3-16) M/E ratios are shown.

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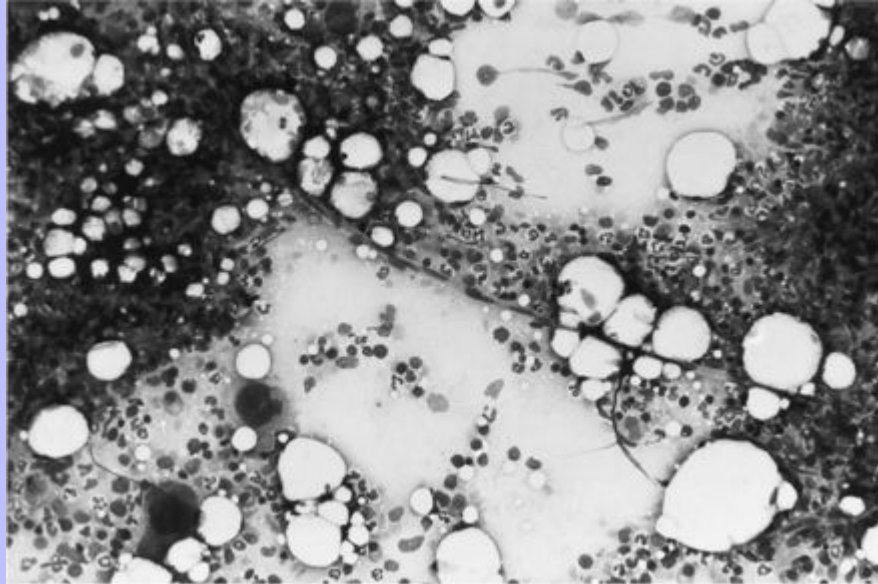
6. Specific comments should be made about lymphocytes, plasma cells, osteoblasts, and osteoclasts when they appear in increased numbers or exhibit abnormal morphology. Small lymphocytes generally account for less than 10% of all nucleated cells in healthy animals, but they may reach 14% in some healthy dogs and 20% in some healthy cats. Ferrets may have considerably higher numbers of marrow lymphocytes than other species. Monocytes, macrophages, and mitotic cells normally do not exceed 1% to 2% of total nucleated cells present. Osteoclasts and osteoblasts are rarely seen except in young, growing animals. Any abnormal cells such as mast cells, neoplastic cells, or macrophages containing phagocytized cells or infective agents should be described. 43
7. The amount of stainable iron present is routinely evaluated by using the Prussian blue reaction. Stainable iron is easily found in normal marrow aspirates from most domestic mammals, as long as particles are present. It is decreased or absent in iron-deficient animals and increased in association with hemolytic anemias and the anemia of chronic disease. Bone marrow iron increases in horses with age; consequently, healthy old horses can have marked amounts of stainable iron present in the marrow. Normal cat bone marrow does not exhibit stainable iron, so its absence cannot be used to confirm a diagnosis of iron deficiency. 44
8. Because reticulocytes are rarely released into blood in response to anemia in horses, reticulocyte counts can be done in bone marrow aspirates from horses to assist in the differential diagnosis of anemia. The presence of more than 5% reticulocytes suggests a regenerative response to anemia. 45
9. The final step in evaluating a bone marrow aspirate is providing an interpretation of the cytologic findings in light of the history, clinical findings, CBC, and results from other diagnostic tests and procedures. For example, a high M/E ratio could indicate the presence of either increased granulocytic cells or decreased erythroid cells. Examination of CBC results from blood collected at the same time usually allows the correct interpretation to be made.

Fig. 3-13



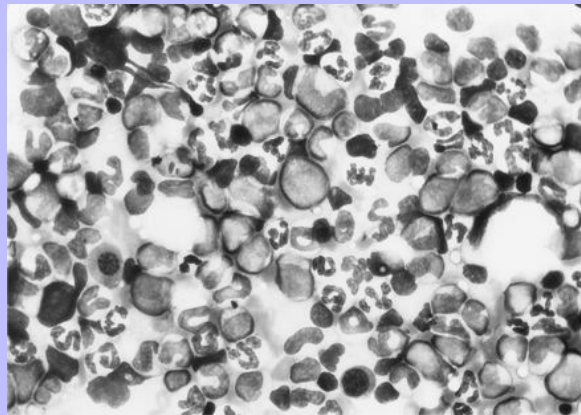
Preparation of a bone marrow smear involves expelling several drops of bone marrow aspirate material onto the end of a glass slide (A). B, Contaminating blood drains down the slide as it is held vertically. C, Adherent marrow particles are squashed between a perpendicularly placed slide, and D, the slides are pulled apart in a horizontal plane in the direction of the arrows. (From Harvey JW: Canine bone marrow: Normal hematopoiesis, biopsy techniques, and cell identification and evaluation. Comp Contin Educ Pract Vet 1984;6:909.)

Fig. 3-14



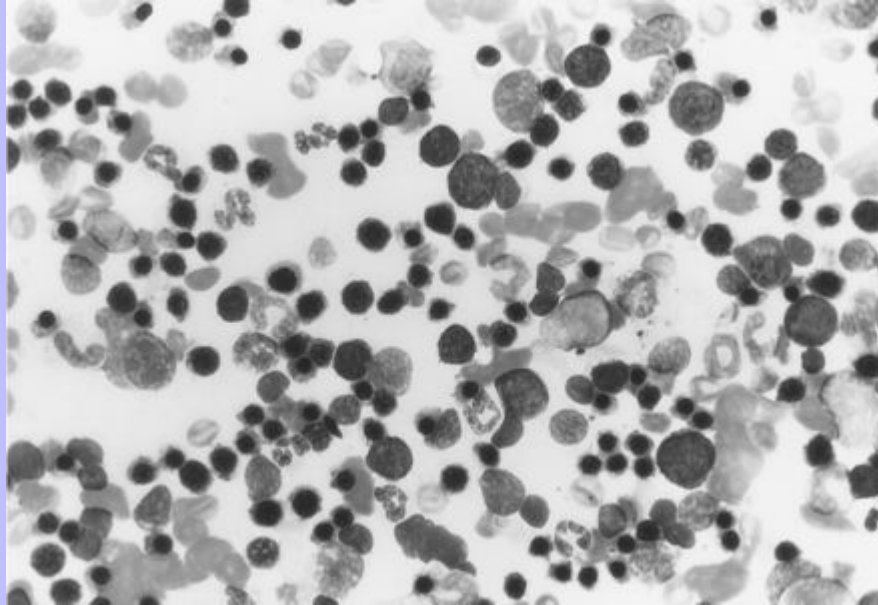
Wright-Giemsa–stained aspirate smear of a dog bone marrow particle with a centrally located capillary. Two megakaryocytes are present (*arrows*). Large vacuoles represent adipocytes from which lipid was removed during fixation. (From Harvey JW: Canine bone marrow: Normal hematopoiesis, biopsy techniques, and cell identification and evaluation. *Comp Contin Educ Pract Vet* 1984;6:909.)

Fig. 3-15



Wright-Giemsa–stained aspirate smear of dog bone marrow with a high myeloid/erythroid ratio. CBC results are needed to determine whether granulocytic hyperplasia or erythroid hypoplasia is present. (From Harvey JW: Canine bone marrow: Normal hematopoiesis, biopsy techniques, and cell identification and evaluation. *Comp Contin Educ Pract Vet* 1984;6:909.)

Fig. 3-16



Wright-Giemsa-stained aspirate smear of dog bone marrow with a low myeloid/erythroid ratio. CBC results are needed to determine whether erythroid hyperplasia or granulocytic hypoplasia is present. (From Harvey JW: Canine bone marrow: Normal hematopoiesis, biopsy techniques, and cell identification and evaluation. *Comp Contin Educ Pract Vet* 1984;6:909.)

### 4.6 ADDITIONAL READING

CN Abboud, MA Lichtman: Structure of the marrow and the hematopoietic microenvironment. In Beutler, E, Lichtman, MA, Coller, BS, Kipps, TJ, Seligsohn, U (Eds.): *Williams hematology*. 6th ed., 2001, McGraw-Hill, New York, 29–58.

JL Abkowitz, D Golinelli, DE Harrison, P Gutter: *In vivo* kinetics of murine hemopoietic stem cells. *Blood*. **96**, 2000, 3399–3405.

GA Ackerman: Developmental relationship between the appearance of lymphocytes and lymphopoietic activity in the thymus and lymph nodes of the fetal cat. *Anat Rec*. **158**, 1967, 387–399.

Y Aiba, F Hirayama, M Ogawa: Clonal proliferation and cytokine requirement of murine progenitors for natural killer cells. *Blood*. **89**, 1997, 4005–4012.

CB Andreasen, TC Gerros, ED Lassen: Evaluation of bone marrow cytology and stainable iron content in healthy adult llamas. *Vet Clin Pathol*. **23**, 1994, 38–42.

PM Appasamy: Biological and clinical implications of interleukin-7 and lymphopoiesis. *Cytokines Cell Mol Ther*. **5**, 1999, 25–39.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- BM Babior, DW Golde: Production, distribution, and fate of neutrophils. In Beutler, E, Lichtman, MA, Coller, BS, Kipps, TJ, Seligsohn, U (Eds.): *Williams hematology*. 6th ed., 2001, McGraw-Hill, New York, 753–759.
- C Campagnoli, IA Roberts, S Kumar, PR Bennett, I Bellantuono, NM Fisk: Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood*. **98**, 2001, 2396–2402.
- M Chang, JX Qian, SM Lee, et al.: Tissue uptake of circulating thrombopoietin is increased in immune-mediated compared with irradiated thrombocytopenic mice. *Blood*. **93**, 1999, 2515–2524.
- P Charbord, R Oostendorp, W Pang, et al.: Comparative study of stromal cell lines derived from embryonic, fetal, and postnatal mouse blood-forming tissues. *Exp Hematol*. **30**, 2002, 1202–1210.
- K Cohen-Solal, N Vitrat, M Titeux, et al.: High-level expression of Mpl in platelets and megakaryocytes is independent of thrombopoietin. *Blood*. **93**, 1999, 2859–2866.
- C Dame, SE Juul: The switch from fetal to adult erythropoiesis. *Clin Perinatol*. **27**, 2000, 507–526.
- R De Maria, U Testa, L Luchetti, et al.: Apoptotic role of Fas/Fas ligand system in the regulation of erythropoiesis. *Blood*. **93**, 1999, 796–803.
- Y Delneste, P Charbonnier, N Herbault, et al.: Interferon-gamma switches monocyte differentiation from dendritic cells to macrophages. *Blood*. **101**, 2003, 143–150.
- JE Dennis, P Charbord: Origin and differentiation of human and murine stroma. *Stem Cells*. **20**, 2002, 205–214.
- MA Duchosal: B-cell development and differentiation. *Semin Hematol*. **34**, 1997, 2–12.
- S Ebbe, MK Boudreaux: Relationship of megakaryocyte ploidy with platelet number and size in cats, dogs, rabbits, and mice. *Comp Haematol Int*. **8**, 1998, 21–25.
- BL Ebert, HF Bunn: Regulation of the erythropoietin gene. *Blood*. **94**, 1999, 1864–1877.
- F El Ouriaghli, H Fujiwara, JJ Melenhorst, et al.: Neutrophil elastase enzymatically antagonizes the *in vitro* action of G-CSF: Implications for the regulation of granulopoiesis. *Blood*. **101**, 2003, 1752–1758.
- JL Galloway, LI Zon: Ontogeny of hematopoiesis: Examining the emergence of hematopoietic cells in the vertebrate embryo. *Curr Top Dev Biol*. **53**, 2003, 139–158.
- MA Goodell: Introduction: Focus on hematology. *CD34(+) or CD34(-): Does it really matter?* *Blood*. **94**, 1999, 2545–2547.
- R Guerriero, G Mattia, U Testa, et al.: Stromal cell-derived factor 1 $\alpha$  increases polyploidization of megakaryocytes generated by human hematopoietic progenitor cells. *Blood*. **97**, 2001, 2587–2595.
- JW Harvey: Canine bone marrow: Normal hematopoiesis, biopsy techniques, and cell identification and evaluation. *Comp Contin Educ Pract Vet*. **6**, 1984, 909–926.
- JW Harvey: Atlas of veterinary hematology. In *Blood and bone marrow of domestic animals*. 2001, WB Saunders, Philadelphia.
- MC Hu, SL Chien: The cytoplasmic domain of stem cell antigen CD34 is essential for cytoadhesion signaling but not sufficient for proliferation signaling. *Blood*. **91**, 1998, 1152–1162.
- G Jego, N Robillard, D Puthier, et al.: Reactive plasmacytoses are expansions of plasmablasts retaining the capacity to differentiate into plasma cells. *Blood*. **94**, 1999, 701–712.
- A Kaser, G Brandacher, W Steurer, et al.: Interleukin-6 stimulates thrombopoiesis through thrombopoietin: Role in inflammatory thrombocytosis. *Blood*. **98**, 2001, 2720–2725.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

M Korbiling, RL Katz, A Khanna, et al.: Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. <i>N Engl J Med.</i> <b>346</b> , 2002, 738–746.	
T Kouro, V Kumar, PW Kincade: Relationships between early B- and NK-lineage lymphocyte precursors in bone marrow. <i>Blood.</i> <b>100</b> , 2002, 3672–3680.	45
T Kouro, KL Medina, K Oritani, et al.: Characteristics of early murine B-lymphocyte precursors and their direct sensitivity to negative regulators. <i>Blood.</i> <b>97</b> , 2001, 2708–2715.	46
DJ Kuter: Megakaryopoiesis and thrombopoiesis. In Beutler, E, Lichtman, MA, Coller, BS, et al. (Eds.): <i>Williams hematology</i> . 6th ed., 2001, McGraw-Hill, New York, 1339–1355.	
WJ Lane, S Dias, K Hattori, et al.: Stromal-derived factor 1-induced megakaryocyte migration and platelet production is dependent on matrix metalloproteinases. <i>Blood.</i> <b>96</b> , 2000, 4152–4159.	
T Lapidot, I Petit: Current understanding of stem cell mobilization: The roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. <i>Exp Hematol.</i> <b>30</b> , 2002, 973–981.	
JM Lean, K Fuller, TJ Chambers: FLT3 ligand can substitute for macrophage colony-stimulating factor in support of osteoclast differentiation and function. <i>Blood.</i> <b>98</b> , 2001, 2707–2713.	
TW LeBien: Fates of human B-cell precursors. <i>Blood.</i> <b>96</b> , 2000, 9–23.	
Q Lin, C Dong, MD Cooper: Impairment of T and B cell development by treatment with a type I interferon. <i>J Exp Med.</i> <b>187</b> , 1998, 79–87.	
SD Lyman, Jacobsen, SEW: <i>c-kit</i> Ligand and flt3 ligand: Stem/progenitor cell factors with overlapping yet distinct activities. <i>Blood.</i> <b>91</b> , 1998, 1101–1134.	
JN MacLeod, JW Tetreault, KA Lorsch, et al.: Expression and bioactivity of recombinant canine erythropoietin. <i>Am J Vet Res.</i> <b>59</b> , 1998, 1144–1148.	
M Majka, A Janowska-Wieczorek, J Ratajczak, et al.: Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. <i>Blood.</i> <b>97</b> , 2001, 3075–3085.	
MG Manz, D Traver, T Miyamoto, et al.: Dendritic cell potentials of early lymphoid and myeloid progenitors. <i>Blood.</i> <b>97</b> , 2001, 3333–3341.	
C Marquez, C Trigueros, JM Franco, et al.: Identification of a common developmental pathway for thymic natural killer cells and dendritic cells. <i>Blood.</i> <b>91</b> , 1998, 2760–2771.	
CJ Marshall, AJ Thrasher: The embryonic origins of human haematopoiesis. <i>Br J Haematol.</i> <b>112</b> , 2001, 838–850.	
S Matsuoka, K Tsuji, H Hisakawa, et al.: Generation of definitive hematopoietic stem cells from murine early yolk sac and paraaortic splanchnopleures by aorta-gonad-mesonephros region-derived stromal cells. <i>Blood.</i> <b>98</b> , 2001, 6–12.	
HK Mikkola, Y Fujiwara, TM Schlaeger, et al.: Expression of CD41 marks the initiation of definitive hematopoiesis in the mouse embryo. <i>Blood.</i> <b>101</b> , 2003, 508–516.	
T Miyamoto, O Ohneda, F Arai, et al.: Bifurcation of osteoclasts and dendritic cells from common progenitors. <i>Blood.</i> <b>98</b> , 2001, 2544–2554.	
R Mohle, F Bautz, C Denzlinger, et al.: Transendothelial migration of hematopoietic progenitor cells. Role of chemotactic factors. <i>Ann N Y Acad Sci.</i> <b>938</b> , 2001, 26–34.	
R Mohle, S Rafii, MA Moore: The role of endothelium in the regulation of hematopoietic stem cell migration. <i>Stem Cells.</i> <b>16</b> (Suppl 1), 1998, 159–165.	

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- O Ohneda, K Ohneda, F Arai, et al.: ALCAM (CD166): Its role in hematopoietic and endothelial development. *Blood*. **98**, 2001, 2134–2142.
- J Palis, MC Yoder: Yolk-sac hematopoiesis: The first blood cells of mouse and man. *Exp Hematol*. **29**, 2001, 927–936.
- DG Phinney: Building a consensus regarding the nature and origin of mesenchymal stem cells. *J Cell Biochem Suppl*. **38**, 2002, 7–12.
- MF Pittenger, AM Mackay, SC Beck, et al.: Multilineage potential of adult human mesenchymal stem cells. *Science*. **284**, 1999, 143–147.
- F Prosper, CM Verfaillie: Regulation of hematopoiesis through adhesion receptors. *J Leukoc Biol*. **69**, 2001, 307–316.
- PJ Quesenberry, GA Colvin: Hematopoietic stem cells, progenitor cells, and cytokines. In Beutler, E, Lichtman, MA, Coller, BS, et al. (Eds.): *Williams hematology*. 6th ed., 2001, McGraw-Hill, New York, 153–182.
- LB Qui, H Dickson, N Hajibagheri, et al.: Extruded erythroblast nuclei are bound and phagocytosed by a novel macrophage receptor. *Blood*. **85**, 1995, 1630–1639.
- M Rais, JS Wild, BK Choudhury, et al.: Interleukin-12 inhibits eosinophil differentiation from bone marrow stem cells in an interferon-gamma-dependent manner in a mouse model of asthma. *Clin Exp Allergy*. **32**, 2002, 627–632.
- Ratcliffe, MJH: B cell development in gut associated lymphoid tissues. *Vet Immunol Immunopathol*. **87**, 2002, 337–340.
- KE Russell, DC Sellon, CB Grindem: Bone marrow in horses: Indications, sample handling, and complications. *Comp Contin Educ Pract Vet*. **16**, 1994, 1359–1365.
- S Sakamaki, Y Hirayama, T Matsunaga, et al.: Transforming growth factor-beta1 (TGF-beta1) induces thrombopoietin from bone marrow stromal cells, which stimulates the expression of TGF-beta receptor on megakaryocytes and, in turn, renders them susceptible to suppression by TGF-beta itself with high specificity. *Blood*. **94**, 1999, 1961–1970.
- T Suda, N Takakura: Role of hematopoietic stem cells in angiogenesis. *Int J Hematol*. **74**, 2001, 266–271.
- K Tiedemann, B van Ooyen: Prenatal hematopoiesis and blood characteristics of the cat. *Anat Embryol (Berl)*. **153**, 1978, 243–267.
- SJ Tornquist, TB Crawford: Suppression of megakaryocyte colony growth by plasma from foals infected with equine infectious anemia virus. *Blood*. **90**, 1997, 2357–2363.
- AM Vannucchi, F Paoletti, S Linari, et al.: Identification and characterization of a bipotent (erythroid and megakaryocytic) cell precursor from the spleen of phenylhydrazine-treated mice. *Blood*. **95**, 2000, 2559–2568.
- A Varas, A Vicente, Sacedón, R, et al.: Interleukin-7 influences the development of thymic dendritic cells. *Blood*. **92**, 1998, 93–100.
- L Wu, A D'Amico, H Hochrein, et al.: Development of thymic and splenic dendritic cell populations from different hemopoietic precursors. *Blood*. **98**, 2001, 3376–3382.
- MC Yoder: Introduction: Spatial origin of murine hematopoietic stem cells. *Blood*. **98**, 2001, 3–5.
- KL Yong, A Fahey, A Pizzey, et al.: Influence of cell cycling and cell division on transendothelial migration of CD34+ cells. *Br J Haematol*. **119**, 2002, 500–509.

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Zamai, L, Secchiero, P, Pierpaoli, S, et al.: TNF-related apoptosis-inducing ligand (TRAIL) as a negative regulator of normal human erythropoiesis. *Blood*. **95**, 2000, 3716–3724.

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## 5 Chapter 4 Evaluation of Erythrocytic Disorders

### 5.1 NORMAL ERYTHROCYTES

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#### 5.1.1 Erythrocyte Morphology

Erythrocytes from most mammals are anucleated biconcave discs called *discocytes*. The biconcave shape results in the central pallor of erythrocytes observed on stained blood films. Among common domestic animals, biconcavity and central pallor are most pronounced in dogs. Erythrocytes from animals in the Camelidae family (camels, llamas, vicunas, alpacas) are anucleated, thin, elliptical cells termed *elliptocytes* (also called *ovalocytes*). They are not biconcave. Erythrocytes from birds, reptiles, and amphibians are also elliptical. They are larger than mammalian erythrocytes and contain nuclei.

#### 5.1.2 Erythrocyte Functions

Mammalian erythrocytes are anucleated cells that normally circulate for several months in blood, despite limited synthetic capacities and repeated exposures to mechanical and metabolic insults. Erythrocytes have three functions: transport of oxygen ( $O_2$ ) to tissue, transport of carbon dioxide ( $CO_2$ ) to the lungs, and buffering of hydrogen ions ( $H^+$ ). In nonanemic animals, the presence of hemoglobin within erythrocytes increases the  $O_2$ -carrying capacity of blood more than 50 times that of plasma without erythrocytes. The  $O_2$  content of blood depends on the blood hemoglobin content, the partial pressure of dissolved oxygen ( $PO_2$ ) in blood, and the affinity of hemoglobin for  $O_2$ .

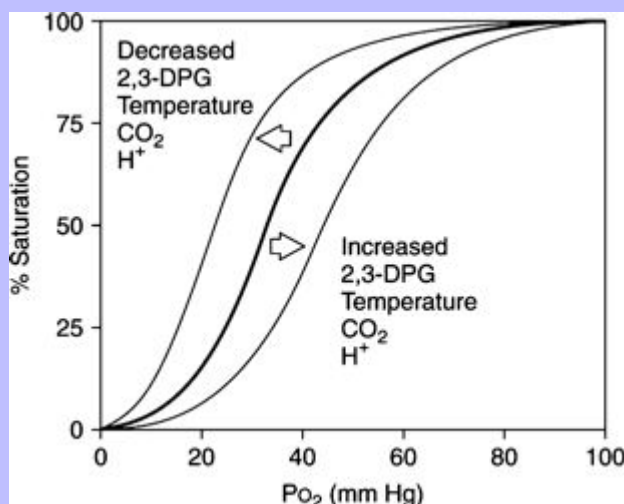
Each hemoglobin tetramer is capable of binding four molecules of  $O_2$  when it is fully oxygenated. The initial binding of a molecule of  $O_2$  to a monomer of tetrameric, deoxygenated hemoglobin facilitates further binding of  $O_2$  to the hemoglobin molecule. The changing  $O_2$  affinity of hemoglobin with oxygenation results in a sigmoid  $O_2$  dissociation curve, (Fig. 4-1) when the percent saturation of hemoglobin with  $O_2$  is plotted against the  $PO_2$ . The steepness of the middle portion of the curve is of great physiologic significance, since it covers the range of  $O_2$  tensions present in tissues. Consequently, a relatively small decrease in  $O_2$  tension results in substantial  $O_2$  release from hemoglobin. The overall affinity of hemoglobin for  $O_2$  is decreased by increasing  $H^+$ ,  $CO_2$ , temperature, and, in most mammals, 2,3-diphosphoglycerate (2,3-DPG). There is a direct correlation between body weight and  $O_2$  affinity of hemoglobin in whole blood (lower body weight, lower  $O_2$  affinity) when various species of mammals are compared.

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Fig. 4-1



Hemoglobin-oxygen dissociation curve and factors influencing the position of the curve. *Decr*, Decrease; 2,3-DPG, 2,3-diphosphoglycerate;  $H^+$ , hydrogen ions; *Incr*, increase;  $PO_2$ , partial pressure of dissolved oxygen. (From Harvey JW: The erythrocyte: Physiology, metabolism and biochemical disorders. In Kaneko JJ, Harvey JW, Bruss ML [eds]: Clinical biochemistry of domestic animals, 5th ed. San Diego, Academic Press, 1997, pp 157-203.)

The  $O_2$  affinity of fetal blood is greater than that of maternal blood, except in the cat. Differences in fetal versus maternal  $O_2$  affinity may potentiate  $O_2$  transport from the mother to the fetus. However, the fetus is subjected to low arterial  $O_2$  tensions, and the increased  $O_2$  affinity of fetal blood is likely needed to more fully saturate hemoglobin with  $O_2$ .

The amount of  $CO_2$  that can be transported dissolved in plasma is small, but the carbonic anhydrase reaction in erythrocytes increases the  $CO_2$ -carrying capacity of blood 17-fold by rapidly converting  $CO_2$  to carbonic acid.

The carbonic acid formed by this reaction spontaneously ionizes to  $H^+$  and bicarbonate ( $HCO_3^-$ ). The bicarbonate diffuses out of the cell down a concentration gradient and chloride ( $Cl^-$ ) moves in (chloride shift) to maintain electrical neutrality. These processes are reversed in the lungs. Some  $CO_2$  is also transported bound to hemoglobin as carbamino groups. Deoxyhemoglobin binds about twice the  $CO_2$  that oxyhemoglobin does.

Hemoglobin is the major protein buffer in blood. Deoxyhemoglobin is a weaker acid than oxyhemoglobin. Consequently, when oxyhemoglobin releases its  $O_2$  in the tissues, the formation of deoxyhemoglobin results in increased binding of  $H^+$ . Hemoglobin buffers the effects of carbonic acid and allows for the isohydric transport of  $CO_2$ . Hemoglobin also buffers organic acids produced during metabolism.

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## 5.1.3 Erythrocyte Biochemistry

The erythrocyte membrane contains a phospholipid bilayer with molecules of unesterified cholesterol intercalated between fatty acid chains. Phospholipids can move in various ways and contribute to membrane fluidity. Glycolipids are located on the outer layer of the membrane, with carbohydrate groups extending outward. Some blood group antigens are glycolipids, with the specificity residing in the carbohydrate moieties (see [Chapter 7](#) for discussion of clinically significant blood groups).

Membranes consist of integral membrane proteins that penetrate the lipid portion, often spanning the bilayer, and skeletal proteins that form or attach to the internal surface of the lipid bilayer. Glycoproteins associated with the membrane are integral membrane proteins, with the carbohydrate residues extending from the outside surface of the cell membrane. They carry erythrocyte antigens and function as receptors or transport proteins (e.g., band 3 is an anion transporter). The membrane skeleton is composed of various proteins located in a lattice-like arrangement on the inner surface of the erythrocyte membrane. This meshwork is attached to the membrane by binding to transmembrane proteins. The membrane skeleton is a major determining factor of membrane shape, deformability, and durability. It is in a condensed configuration in intact cells and can be stretched considerably without rupturing.

Erythrocytes in mammals lack nuclei. Therefore they cannot synthesize DNA or RNA. They also lack ribosomes, mitochondria, and endoplasmic reticulum and, consequently, have no Krebs cycle or electron transport system and are unable to synthesize proteins or lipids (de novo). Mature erythrocytes depend on anaerobic glycolysis for energy ([Fig. 4-2](#)). Adenosine triphosphate (ATP) is needed for maintenance of erythrocyte ionic composition, for maintenance of erythrocyte shape and deformability, and for limited synthetic activities such as glutathione synthesis. Erythrocyte 2,3-DPG is produced from a side pathway of the anaerobic glycolysis pathway. No net ATP is generated when molecules traverse this pathway. The formation of 2,3-DPG in erythrocytes is stimulated by increased blood inorganic phosphate concentration and increased blood pH, which both stimulate anaerobic glycolysis in erythrocytes. 2,3-DPG is the most abundant organic phosphate in erythrocytes of most species, but the concentration of 2,3-DPG is low in erythrocytes of cats and mature ruminants. Animals with high erythrocyte 2,3-DPG concentrations, such as dogs and horses, have the potential to alter their hemoglobin O<sub>2</sub> affinity to meet metabolic needs. The significance of (and in some cases the appropriateness of) alterations in 2,3-DPG concentration in disease states is not always clear. The erythrocyte 2,3-DPG concentration increases in some anemic animals, and the resultant decrease in hemoglobin O<sub>2</sub> affinity would seem to be beneficial.

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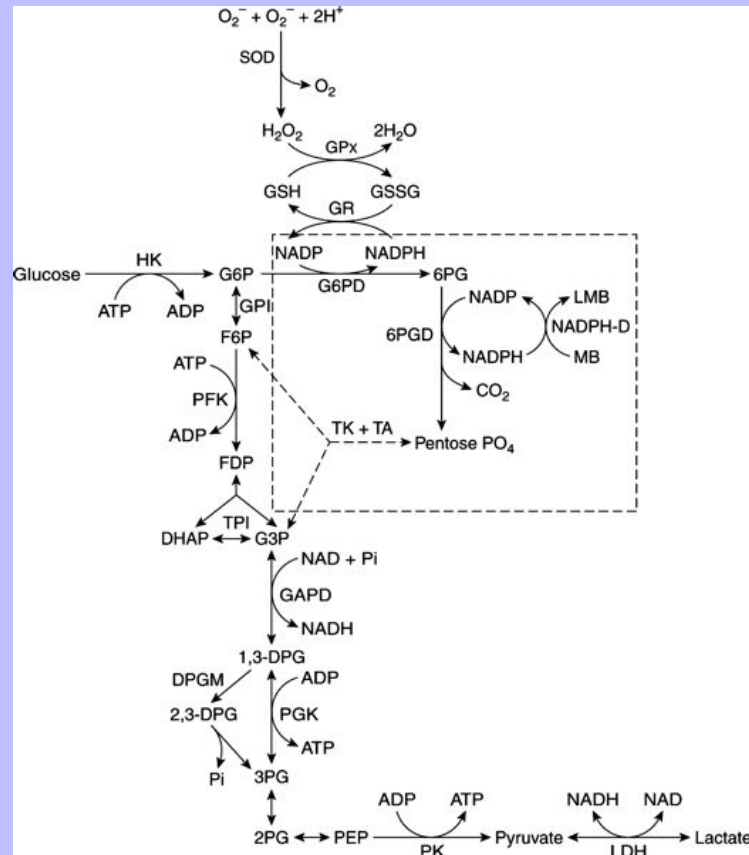
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Fig. 4-2



Metabolic pathways of the mature erythrocyte. *ADP*, Adenosine diphosphate; *ATP*, adenosine triphosphate; *DHAP*, dihydroxyacetone phosphate; *1,3DPG*, 1,3-diphosphoglycerate; *2,3-DPG*, 2,3-diphosphoglycerate; *DPGM*, diphosphoglycerate mutase; *FDP*, fructose 1,6-diphosphate; *F6P*, fructose 6-phosphate; *GAP*, glyceraldehyde 3-phosphate; *GAPD*, glyceraldehyde-3-phosphate dehydrogenase; *G6P*, glucose 6-phosphate; *G6PD*, glucose-6-phosphate dehydrogenase; *GPI*, glucose phosphate isomerase; *GPx*, glutathione peroxidase; *GR*, glutathione reductase; *GSH*, reduced glutathione; *GSSG*, oxidized glutathione; *HK*, hexokinase; *H<sub>2</sub>O<sub>2</sub>*, hydrogen peroxide; *LDH*, lactate dehydrogenase; *LMB*, leukomethylene blue; *MB*, methylene blue; *MPGM*, monophosphoglycerate mutase; *NAD*, nicotinamide adenine dinucleotide; *NADH*, reduced nicotinamide adenine dinucleotide; *NADP*, nicotinamide adenine dinucleotide phosphate; *NADPH*, reduced nicotinamide adenine dinucleotide phosphate; *NADPH-D*, reduced nicotinamide adenine dinucleotide phosphate diaphorase; *PEP*, phosphoenolpyruvate; *PFK*, phosphofructokinase; *2PG*, 2-phosphoglycerate; *3PG*, 3-phosphoglycerate; *6PGD*, 6-phosphogluconate dehydrogenase; *PGK*, phosphoglycerate kinase; *P<sub>i</sub>*, inorganic phosphate; *PK*, pyruvate kinase; *SOD*, superoxide dismutase; *TK*, transketolase; *TA*, transaldolase; *TPI*, triosephosphate isomerase. (From Harvey JW: The erythrocyte: Physiology, metabolism and biochemical disorders. In Kaneko JJ, Harvey JW, Bruss ML [eds]: Clinical biochemistry of domestic animals, 5th ed. San Diego, Academic Press, 1997, pp 157-203.)

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Oxidative reactions can damage hemoglobin, enzymes (sulfhydryl groups especially), and membrane lipids. Methemoglobin forms when hemoglobin iron is oxidized from the +2 to the +3 state. Heinz bodies are inclusions that form within erythrocytes after the oxidative denaturation of the globin portion of hemoglobin. Oxidative membrane damage can result in intravascular hemolysis or erythrophagocytosis and shortened erythrocyte life spans.

Reduced nicotinamide adenine dinucleotide phosphate (NADPH), generated in the pentose phosphate pathway, provides electrons for protection against oxidants. Reduced glutathione (GSH) is a tripeptide containing one sulfhydryl group. GSH is a substrate for the glutathione peroxidase reaction, in which it provides electrons for the reduction of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Glutathione peroxidase contains selenium, which accounts for some of selenium's antioxidant properties. GSH can also react directly with various free radicals. Oxidized glutathione can be reduced back to GSH molecules by the glutathione reductase enzyme with NADPH as the source of electrons (see Fig. 4-2). Catalase is an enzyme that can catalyze the conversion of hydrogen peroxide to water and  $\text{O}_2$  without using energy. Vitamin E is lipid soluble and acts as a free radical scavenger in the membrane.

About 3% of hemoglobin ( $\text{Fe}^{+2}$ ) is oxidized to methemoglobin ( $\text{Fe}^{+3}$ ) each day. Methemoglobin is unable to bind  $\text{O}_2$ . To prevent hypoxia that would result from the accumulation of a high level of methemoglobin, the methemoglobin formed is reduced back to functional hemoglobin in a reaction that requires the methemoglobin reductase (cytochrome- $b_5$  reductase) enzyme and reduced nicotinamide adenine dinucleotide (NADH) generated by anaerobic glycolysis.

## 5.1.4 Iron Metabolism

More iron is needed for erythrocyte production than for production of all other cells in the body combined. Developing erythroid cells generally extract 70% to 95% of the iron circulating in plasma, and 55% to 65% of the iron in the body occurs in hemoglobin within erythroid cells (3.4 mg iron per gram of hemoglobin).

Iron absorption depends on age, iron status, and health of the animal, as well as the amount and chemical form of iron ingested. Three pathways of iron absorption have been identified, with separate pathways utilized for ferric iron ( $\text{Fe}^{+3}$ ), ferrous iron ( $\text{Fe}^{+2}$ ), and iron contained within heme. The relative importance of these pathways varies depending on animal species and diet.

Most inorganic iron in the diet is in the ferric state. Ferric iron is solubilized from food by hydrochloric acid in the stomach and binds to mucins and various small molecules in the stomach, which keep the iron soluble and available for absorption in the more alkaline environment of the small intestine. Ferric iron molecules enter duodenal and jejunal mucosal cells by binding to  $\beta_3$ -integrin molecules on their luminal surfaces and combining with a cytoplasmic shuttle protein called *mobilferrin*. Within the cytoplasm, the integrin-mobilferrin-iron complex combines with flavin monooxygenase and other constituents to form a large complex called *paraferitin*. This complex serves as a ferrireductase to reduce ferric iron to the ferrous state used in heme synthesis.

After reduction of ferric iron by a brush border ferrireductase, ferrous iron absorption is facilitated by divalent metal transporter 1 (DMT1) through a proton-coupled process. This transporter is not specific for iron because it can transport manganese and other divalent metal ions.

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Heme is released from dietary myoglobin and hemoglobin by the action of digestive enzymes and probably enters the mucosal cell as an intact metalloporphyrin by endocytosis. Once inside the cell, inorganic iron is released from heme by the action of the heme oxygenase reaction.

The mechanism of iron transfer from mucosal cells to transferrin in plasma is less well defined. An iron transport protein (variably named *ferroportin1*, *Ireg1*, *MTP1*) has recently been identified on the basolateral surface of mature duodenal mucosal cells and liver Kupffer cells, and it appears to be important in the export of iron from these cells. The release of iron from mucosal cells is also facilitated by a copper-containing protein called *hephaestin*, which is located on the basolateral membranes. *Hephaestin* exhibits ferroxidase activity and has significant homology to ceruloplasmin.

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The mucosal cell protects itself against the toxic effects of free iron by increasing apoferritin synthesis and incorporating the excess iron into ferritin. Each ferritin molecule is composed of a protein shell of 24 apoferritin subunits surrounding a central core of up to 4500 iron atoms as ferric oxyhydroxide. Ferritin is a storage protein that prevents free iron from catalyzing oxidative reactions, which would injure the cell. Ferritin within mucosal cells is lost when these cells are shed into the intestinal lumen. Normally, only a small percentage of dietary iron enters the circulation. Iron absorption is increased when total body iron content is low, erythropoiesis is increased, or hypoxia is present. Iron absorption is decreased when total body iron content is high.

Hepcidin, a peptide produced by hepatocytes, appears to be an important negative regulator of iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages. Hepcidin synthesis is stimulated by inflammation (interleukin 6–mediated) or iron overload.

As indicated earlier, more than half of the total body iron is present in hemoglobin. About a third is stored as ferritin and hemosiderin (primarily within macrophages), 3% to 7% is present in myoglobin (with the higher values occurring in dogs and horses), 1% is present in hemoprotein and flavoprotein enzymes, and only 0.1% is bound to transferrin in plasma.

Free cytoplasmic ferritin molecules are visible by electron microscopy, but not by light microscopy. Hemosiderin is composed of aggregates of protein and iron within lysosomes. It is insoluble in water and thought to result from the degradation of ferritin. Hemosiderin is visible by light microscopy when stained with an iron stain (Prussian blue stain). Iron stored as hemosiderin is released more slowly for binding to transferrin in plasma than iron stored as ferritin.

Transferrin is a  $\beta$ -globulin with two binding sites for  $\text{Fe}^{+3}$ . Normally, 25% to 50% of the plasma transferrin binding sites are saturated with iron. Although ferritin is released in small amounts from macrophages and can be taken up by cells (especially hepatocytes), most iron is transported to developing erythroid cells by transferrin. Transferrin molecules bind to transferrin receptors (TfRs) on the surface of cells. After binding, the transferrin-TfR complex is internalized by endocytosis. A proton pump decreases the pH in the endosome, resulting in conformational changes in the proteins and subsequent release of iron from transferrin. The resultant apotransferrin-TfR complex is recycled to the cell membrane where apotransferrin is released from the cell, and the TfR is again available for binding additional iron-containing transferrin molecules. After release from transferrin, iron is transported to mitochondria, where iron is incorporated into protoporphyrin to form heme. The nature of this intracellular transport is unclear. Iron may be bound to mobilferrin, complexed with low-molecular-weight compounds, or directly delivered to mitochondria by transient mitochondria-endosome interactions. TfR and apoferritin synthesis are regulated by the amount of intracellular iron present. High iron content stimulates apoferritin synthesis and inhibits TfR expression to minimize the potential for iron toxicity to the cell. Low iron content results in decreased apoferritin synthesis and increased TfR expression on cell surfaces.

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to maximize iron uptake and use for heme synthesis. Free heme concentration within erythroid cells controls hemoglobin synthesis. An increase in free heme promotes the synthesis of globin chains and inhibits the uptake of iron from transferrin.

## 5.2 ERYTHROCYTE DESTRUCTION

### 5.2.1 Normal Removal of Aged Erythrocytes

Most erythrocytes circulate in blood for a finite period (survival time or life span), which ranges from 2 to 5 months in domestic animals, depending on the species. Erythrocyte life spans are related to body weight (and consequently metabolic rate), with the smallest animals (highest metabolic rate) having the shortest erythrocyte life spans. Aged erythrocytes are phagocytized by the mononuclear phagocyte system. A variety of changes occur in erythrocytes as they get older. Current evidence suggests that cumulative oxidative injury to membrane components is responsible for normal erythrocyte aging and removal.

Surface membrane alterations on aged or damaged cells that may be recognized by macrophages include the loss of membrane phospholipid asymmetry, altered carbohydrate moieties of membrane proteins, and adherence of anti-band 3 autoantibodies. Phosphatidylserine is normally localized in the inner leaflet of the lipid bilayer, but with cell damage, phosphatidylserine may be exposed on the outer leaflet of the lipid bilayer, where it can be bound by macrophage scavenger receptors. Other macrophage receptors can recognize altered carbohydrate moieties on the surface of erythrocytes. Oxidative damage to erythrocytes results in the clustering of band 3 to form a senescent antigen. Macrophages may directly recognize the altered carbohydrate chains attached to band 3, but a natural antibody in plasma also binds to senescent cell antigens on the surface of aged cells. This autoantibody against clustered band 3, together with bound complement, promotes the phagocytosis of aged erythrocytes by macrophages that exhibit Fc and C3b surface receptors. The relative importance of these various pathways in the recognition and removal of aged erythrocytes remains to be clarified.

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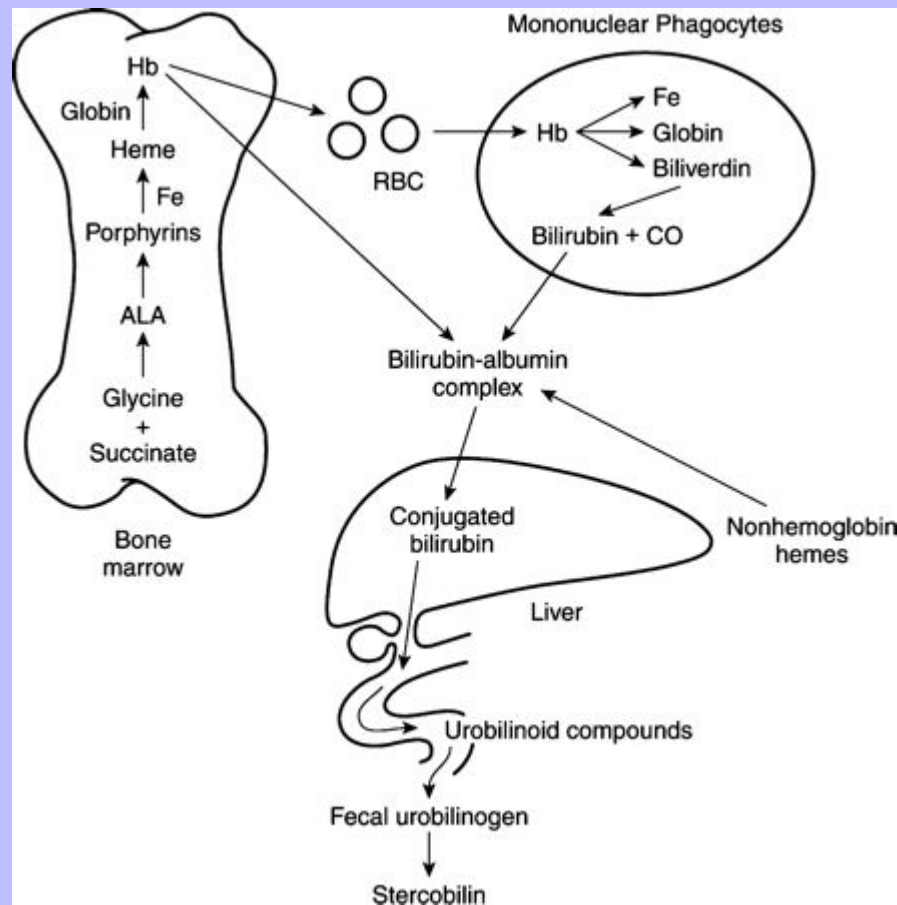
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After phagocytosis by macrophages of the spleen and other organs of the mononuclear phagocyte system, erythrocytes are lysed, and hemoglobin is degraded to heme and globin (Fig. 4-3). Globin is catabolized to constituent amino acids, and the microsomal heme oxygenase reaction within macrophages degrades heme to iron, carbon monoxide, and biliverdin. Iron is conserved and stored as ferritin and hemosiderin in the macrophages. It can be released to the circulation and transported back to developing erythroid cells bound to transferrin. Biliverdin is reduced to bilirubin via biliverdin reductase. Bilirubin is then released from the macrophage and bound to albumin for transport to the liver for conjugation and excretion. Approximately 85% of the bilirubin produced in the body comes from the degradation of hemoglobin, with the remaining percentage coming from the degradation of other heme-containing proteins.

Fig. 4-3



Overview of erythrocyte production, erythrocyte phagocytosis by mononuclear phagocytes, hemoglobin degradation, and bilirubin metabolism. ALA, Aminolevulinic acid; Hb, hemoglobin; RBC, red blood cells; Fe, iron; CO, carbon monoxide.

### 5.2.2 Pathologic Destruction of Erythrocytes

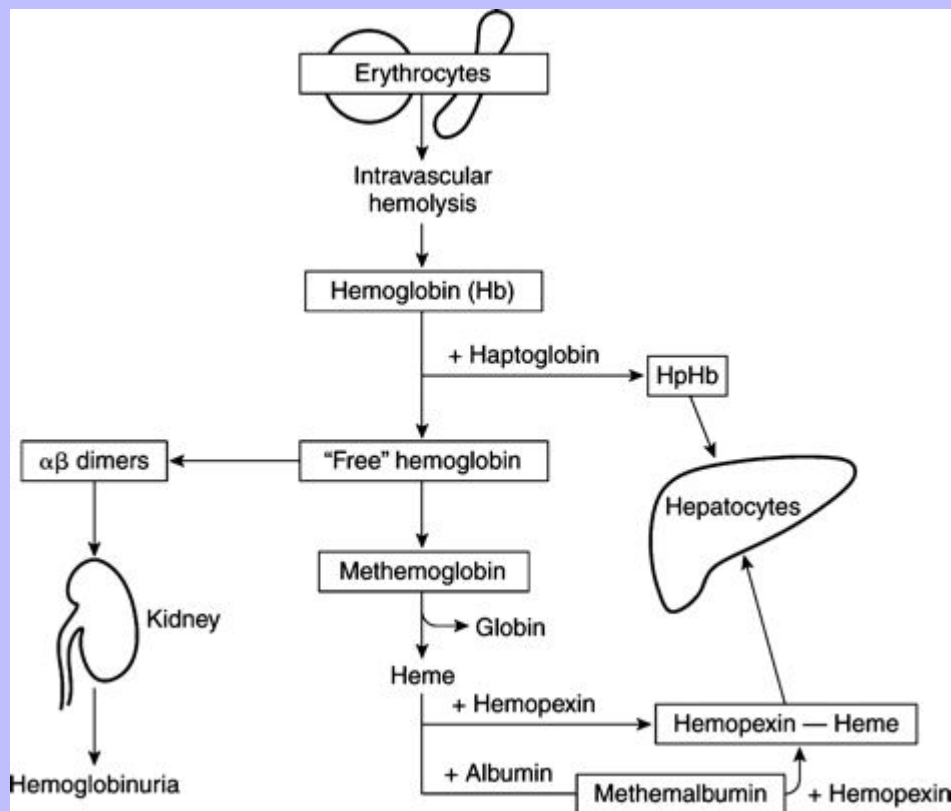
Increased membrane injury associated with pathologic disorders can result in increased phagocytosis of erythrocytes. Anemia develops if the rate of destruction exceeds the ability of the bone marrow to respond by producing new erythrocytes. Lysis of erythrocytes within macrophages is sometimes referred to as *extravascular hemolysis*. Hyperbilirubinemia may be present within a few hours after substantial erythrocyte destruction.

Almost no lysis of erythrocytes occurs within the circulation in healthy individuals, but intravascular hemolysis can be present when severe membrane damage occurs in disease states (Fig. 4-4). After lysis, hemoglobin in plasma (hemoglobinemia) reversibly dissociates into dimers that bind nearly irreversibly to haptoglobin, an  $\alpha_2$ -glycoprotein. The hemoglobin-haptoglobin complex is too large to be filtered through the kidneys and is

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removed from the circulation after binding to receptors on macrophages and hepatocytes. The hemoglobin is degraded and iron is conserved. Once plasma haptoglobin is saturated (about 50 to 150 mg/dL hemoglobin-binding capacity in dogs, cats, and horses), remaining free hemoglobin dimers are small enough to be readily filtered by the kidneys. Some hemoglobin is reabsorbed by the proximal tubules, but once that capacity is exceeded, hemoglobin appears in the urine (hemoglobinuria). Plasma appears red when as little as 50 mg/dL hemoglobin is present; consequently, hemoglobinemia may be observed in the absence of hemoglobinuria. Hemoglobin absorbed by the proximal tubules is rapidly catabolized, and iron is stored as ferritin and hemosiderin. Iron not reutilized is lost when tubular epithelial cells slough into the urine, producing hemosiderinuria.

Fig. 4-4



Pathophysiology of intravascular hemolysis. Methemalbumin forms in primates, but not in common domestic animals.

Free hemoglobin in plasma can spontaneously oxidize to form methemoglobin, which tends to dissociate into heme (ferriheme) and globin. Free heme binds to a plasma  $\beta$ -globulin called *hemopexin* for transport to the liver. The binding to hemopexin protects cell membranes from toxic effects of free heme, and it also conserves iron. Albumin from primates can also bind heme to form methemalbumin, but albumin from common domestic animals does not bind heme.

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## 5.3 EVALUATION OF ERYTHROCYTES

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Erythrocytes in blood are quantified by cell counting (erythrocytes per microliter), by determining blood hemoglobin content (in grams per deciliter), and by determining the hematocrit (HCT) or packed cell volume as a percentage of blood volume. Because essentially all hemoglobin is present within erythrocytes, the erythrocyte count or red blood cell (RBC) count, HCT, and hemoglobin content parallel each other when a change occurs. The HCT can be measured by centrifugation of blood in a microhematocrit tube. When measured in this way, the HCT is the easiest and most reproducible test available for quantifying erythrocytes in clinical practice. The RBC count and hemoglobin content need only be measured when erythrocyte indices are to be calculated.

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The RBC count and mean cell volume (MCV) are accurate if they are measured with an electronic cell counter that has been designed or adjusted to measure the variably sized erythrocytes of animals. The hemoglobin content is measured spectrophotometrically with the cyanmethemoglobin method in most automated systems. Modern electronic cell counters calculate the HCT by using the measured RBC count and MCV. This efficiency negates the need to centrifuge a microhematocrit tube of blood. Unfortunately, useful information concerning the appearance of plasma is missed when the HCT is determined electronically, unless a serum sample is also prepared for clinical chemistry tests. The HCT determined by centrifugation of blood in a microhematocrit tube may be 1 to 3 percentage points higher than the electronically calculated value because of trapped plasma in the centrifuged sample.

### 5.3.1 BOX 4-1 Concomitant Interpretation of Hematocrit (HCT) and Total Plasma Protein (TPP) Concentration

#### 5.3.1.1 Normal HCT with:

Low TPP—Gastrointestinal protein loss, proteinuria, severe liver disease, vasculitis

Normal TPP—Normal

High TPP—Increased globulin synthesis, dehydration-masked anemia

#### 5.3.1.2 High HCT with:

Low TPP—A combination of splenic contraction and a source of protein loss

Normal TPP—Splenic contraction, primary or secondary erythrocytosis, dehydration-masked hypoproteinemia

High TPP—Dehydration

#### 5.3.1.3 Low HCT with:

Low TPP—Substantial ongoing or recent blood loss, overhydration

Normal TPP—Increased erythrocyte destruction, decreased erythrocyte production, chronic blood loss

High TPP—Anemia of inflammatory disease, multiple myeloma, lymphoproliferative diseases

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The capacity of the spleen to expand and contract can result in substantial changes in the HCT, especially in horses and dogs. Excitement or exercise with splenic contraction immediately before phlebotomy can result in 30%, 40%, and 50% increases in the HCT of cats, dogs, and horses, respectively. Conversely, anesthesia (especially with barbiturates) can cause splenic enlargement, and the HCT may drop below the reference interval.

Maximum information can be obtained by interpretation of the HCT and plasma protein concentrations simultaneously. Various combinations of low, normal, and high HCT values may occur with low, normal, or high plasma protein concentrations. The various combinations and examples of how they can be interpreted are given in [Box 4-1](#).

## 5.3.2 Abnormal Erythrocyte Morphology

In addition to the material presented in this text, the reader is referred to an atlas by Harvey (2001) for many color images demonstrating abnormal erythrocyte morphology, as well as a detailed list of references.

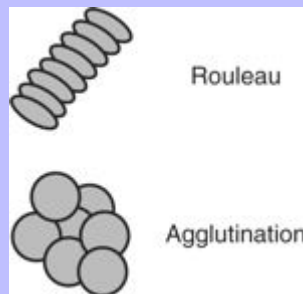
### 5.3.2.1 Rouleau Formation

Erythrocytes on blood films from healthy horses and cats often exhibit rouleau formation (adhesion of erythrocytes together like a stack of coins) ([Fig. 4-5](#)). Increased concentrations of fibrinogen and globulin proteins potentiate rouleau formation in association with inflammatory conditions. Rouleau formation can also occur in association with some lymphoproliferative disorders in which one or more immunoglobulins are secreted in large amounts. Prominent rouleau formation in species other than horses and cats should be noted as an abnormal finding.

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Fig. 4-5



The pattern of erythrocyte adhesion that occurs with rouleau is compared with the pattern that occurs with agglutination.

### 5.3.2.2 Agglutination

The aggregation or clumping of erythrocytes together in clusters (not chains like rouleaux) is termed *agglutination* (see [Fig. 4-5](#)). It is caused by the binding of immunoglobulins to erythrocyte surfaces. Because of their pentavalent nature, IgM immunoglobulins have the greatest propensity to produce agglutination. High-dose unfractionated heparin treatment in horses also causes erythrocyte agglutination by an undefined mechanism.

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5.3.2.3

## Anisocytosis

Variation in erythrocyte diameters on stained blood films is called *anisocytosis* (Plates 1 and 4). It is greater in healthy cattle than in other healthy domestic animals. Anisocytosis is increased when different populations of cells are present. Increased anisocytosis may occur when substantial numbers of smaller than normal cells are produced, as occurs with iron deficiency, or when substantial numbers of larger than normal cells are produced, as occurs when increased numbers of reticulocytes are formed. Consequently, increased anisocytosis is usually present in regenerative anemia, but it may be present in some nonregenerative anemias resulting from dyserythropoiesis. Anisocytosis, without polychromasia, may be seen in horses with intensely regenerative anemia.

5.3.2.4

## Polychromasia

The presence of bluish red erythrocytes on stained blood films is called *polychromasia* (Plate 1). Polychromatophilic erythrocytes are reticulocytes that stain bluish red because of the combined presence of hemoglobin (red-staining) and ribosomes (blue-staining). Low numbers of polychromatophilic erythrocytes are usually seen in blood from healthy dogs and pigs because up to 1.5% reticulocytes may be present in dogs and up to 1% reticulocytes may be present in pigs even when the HCT is normal. Slight polychromasia may be present in healthy cats, but it is usually absent. Polychromasia is absent on stained blood films from healthy cattle, sheep, goats, and horses because reticulocytes are not normally present in blood of these species. There is a direct correlation between the degree of polychromasia and reticulocytosis in dogs (and presumably in pigs) and aggregate reticulocyte percentages in cats. The presence of increased polychromasia in anemic animals indicates that an increased percentage of reticulocytes is present. Polychromasia is not present during regenerative anemia in horses because horses rarely release reticulocytes from the bone marrow.

5.3.2.5

## Hypochromasia

The presence of erythrocytes with decreased hemoglobin concentration and increased central pallor is called *hypochromasia* (Plate 3). Not only is the center of the cell paler than normal, but the diameter of the area of central pallor is increased relative to the red-staining periphery of the cell. True hypochromic erythrocytes must be differentiated from torocytes, which have colorless, punched-out centers but wide, dense red-staining peripheries. Torocytes are generally artifacts. Increased hypochromasia is observed in dogs and ruminants with iron deficiency anemia because the iron-deficient erythrocytes are thin cells (leptocytes) containing decreased hemoglobin concentration.

5.3.2.6

## Poikilocytosis

Poikilocytosis is a general term used to describe the presence of abnormally shaped erythrocytes (Fig. 4-6).

Poikilocytosis may be present in clinically healthy goats and young cattle. In some instances, these shapes appear to be related to the hemoglobin types present. Dogs and ruminants with severe iron deficiency anemia may have pronounced poikilocytosis, the etiology of which is unknown. Poikilocytes can form when oxidant injury results in Heinz body formation and/or membrane injury. One or more blunt erythrocyte surface projections may form as the membrane adheres to Heinz bodies bound to its internal surface. A variety of abnormal erythrocyte shapes have been reported in dogs and cats with doxorubicin toxicity and in animals with dyserythropoiesis. More specific terminology is used for certain abnormal shapes.

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Fig. 4-6



Erythrocyte shapes. See the text for causes and significance.

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## 5.3.2.7 Echinocytes (Crenated Erythrocytes)

Echinocytes are spiculated erythrocytes in which the spicules are relatively evenly spaced and of similar size. Spicules may be sharp or blunt. When observed on stained blood films, echinocytosis is usually an artifact that results from excess ethylenediaminetetraacetic acid (EDTA), improper smear preparation, or prolonged sample storage before blood film preparation. They are common in normal pig blood smears, forming when erythrocytes contact glass surfaces. Echinocytes form when the surface area of the outer lipid monolayer increases relative to the inner monolayer. Echinocytic transformation occurs in vitro in the presence of fatty acids, lysophospholipids, and amphipathic drugs that distribute preferentially in the outer half of the lipid bilayer. Echinocytes also form when erythrocytes are dehydrated, pH is increased, erythrocyte ATP is depleted (e.g., hypophosphatemia), and intracellular calcium is increased. Transient echinocytosis occurs in dogs after coral snake and rattlesnake envenomation, presumably as a result of the action of phospholipases present in venom. Depending on the time course and dose of venom received, either echinocytosis or spherocytosis may be observed after these snakebites. Echinocytes may occur in uremic animals, immediately after transfusion of stored blood, or in some pyruvate kinase-deficient dogs. They have been seen with increased frequency in dogs with glomerulonephritis, neoplasia (lymphoma, hemangiosarcoma, mast cell tumor, and carcinoma), and doxorubicin treatment. Echinocytosis occurs in horses when total body depletion of cations has occurred (endurance exercise, furosemide treatment, diarrhea, systemic disease).

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## 5.3.2.8 Acanthocytes

Erythrocytes with irregularly spaced, variably sized spicules are called *acanthocytes*. They form when erythrocyte membranes contain excess cholesterol compared with phospholipids. Alterations in erythrocyte membrane lipids can result from increased blood cholesterol content or the presence of abnormal plasma lipoprotein composition. Acanthocytes have been recognized in animals with liver disease, where acanthocyte formation may be caused by alterations in plasma lipid composition, which can alter erythrocyte lipid composition. Acanthocytes have also been reported in dogs with disorders that result in erythrocyte fragmentation, such as hemangiosarcoma, disseminated intravascular coagulation, and glomerulonephritis.

Marked acanthocytosis is reported to occur in young goats and some young cattle. Acanthocytosis of young goats occurs as a result of the presence of hemoglobin C (HbC) at this early stage of development.

## 5.3.2.9 Keratocytes

Erythrocytes containing what appears to be one or more intact or ruptured “vesicles” are called *keratocytes*. These nonstaining areas appear to be circular areas of apposed and sealed membrane rather than true vesicles. Removal or rupture of this area results in the formation of one or two projections. Keratocytes have been recognized in various disorders including iron deficiency anemia, liver disorders, and doxorubicin toxicity in cats and in various disorders in dogs that have concomitant echinocytosis or acanthocytosis. Keratocyte formation is potentiated by storage of cat blood collected with EDTA.

## 5.3.2.10 Stomatocytes

Cup-shaped erythrocytes that have oval or elongated areas of central pallor when viewed on stained blood films are called *stomatocytes*. They most often occur as artifacts in thick blood film preparations. Stomatocytes form when erythrocyte water content is increased, as occurs in hereditary stomatocytosis in

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dogs. Stomatocytes also form when amphipathic drugs that distribute preferentially in the inner half of the lipid bilayer are present.

## 5.3.2.11 Spherocytes

Spherical erythrocytes that result from cell swelling and/or loss of cell membrane are referred to as *spherocytes*. Spherocytes lack central pallor and have smaller diameters than normal on stained blood films. Spherocytes occur most frequently in association with immune-mediated hemolytic anemia in dogs. Other potential causes of spherocyte formation include coral snake and rattlesnake envenomation, bee stings, zinc toxicity, erythrocyte parasites, transfusion of stored blood, and a familial dyserythropoiesis in dogs. Since erythrocytes from other common domestic animals exhibit less central pallor than those of dogs, it is difficult to be certain when spherocytes are present in these noncanine species. Hereditary spherocytosis has been reported in Japanese Black cattle with erythrocyte band 3 deficiency and in golden retrievers with a partial spectrin deficiency in erythrocyte membranes.

## 5.3.2.12 Schistocytes

Erythrocyte fragmentation may occur when erythrocytes are forced to flow through altered vascular channels or are exposed to turbulent blood flow. Erythrocyte fragments with two or three pointed extremities are called *schistocytes* or *schizocytes*. Schistocytes are smaller than normal discocytes. Schistocytes may be seen in dogs with microangiopathic hemolytic anemia associated with disseminated intravascular coagulation. Mechanical fragmentation occurs as the cells pass through the fibrin meshwork of a microthrombus. Schistocytes are not typically seen in cats and horses with disseminated intravascular coagulation, possibly because the erythrocytes of these species are smaller and less likely to be split by fibrin strands in the circulation. Schistocytes have also been seen in severe iron deficiency anemia, myelofibrosis, liver disease, congestive heart failure, glomerulonephritis, hemophagocytic histiocytic disorders, hemangiosarcoma, and congenital and acquired dyserythropoiesis in dogs. Marked poikilocytosis with schistocytes and acanthocytes has been recognized in pyruvate kinase-deficient dogs after splenectomy. It is assumed that the spleen had previously removed these fragmented erythrocytes.

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## 5.3.2.13 Leptocytes

Leptocytes are thin, flat, hypochromic appearing erythrocytes with increased membrane to volume ratios. Some leptocytes appear folded, some appear as triconcave knizocytes that give the impression that the erythrocyte has a central bar of hemoglobin, and others appear as codocytes. Codocytes (target cells) are bell-shaped cells that exhibit a central density or “bull’s-eye” on stained blood films. Small numbers of codocytes are often seen in normal dog blood, and both codocytes and knizocytes are increased in dogs with regenerative anemia. Codocytes are especially increased in dogs with congenital dyserythropoiesis. Leptocytes may be seen in iron deficiency anemia and rarely in hepatic insufficiency that results in a balanced accumulation of membrane phospholipids and cholesterol. Polychromatophilic erythrocytes can sometimes appear as leptocytes.

## 5.3.2.14 Eccentrocytes

An erythrocyte in which the hemoglobin is localized to part of the cell, leaving a hemoglobin-poor area visible in the remaining part of the cell, is termed an *eccentrocyte*. This cell shape is formed by the adhesion of opposing areas of the cytoplasmic face of the erythrocyte membrane. Eccentrocytes that have become

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spherical with only a small tag of cytoplasm remaining may be called *pyknocytes*. Eccentrocytes have been seen in the blood of animals ingesting or receiving oxidants, including onions, garlic, acetaminophen, and vitamin K (dogs); red maple leaves (horses); and intravenous hydrogen peroxide as a “home remedy” (a cow). Eccentrocytes have also been seen in a horse with glucose-6-phosphate dehydrogenase deficiency and in horses with glutathione reductase deficiency associated with erythrocyte flavin adenine dinucleotide deficiency.

## 5.3.2.15 Elliptocytes (Ovalocytes)

Nonmammals and animals in the Camelidae family normally have erythrocytes that are elliptical or oval. They are generally flat rather than biconcave. Abnormal elliptocytes have been detected in the blood of cats with bone marrow abnormalities (myeloproliferative disorders and acute lymphoblastic leukemia), hepatic lipidosis, portosystemic shunts, and doxorubicin toxicity and in the blood of dogs with myelofibrosis, myelodysplastic syndrome, and glomerulonephritis. Hereditary elliptocytosis has been reported in a dog with a membrane protein 4.1 deficiency.

## 5.3.2.16 Drepanocytes (Sickle Cells)

Fusiform or spindle-shaped erythrocytes are often observed in blood from healthy deer and in blood from humans with sickle cell anemia. Drepanocytes in deer develop as a result of hemoglobin polymerization, which is an in vitro phenomenon that occurs when O<sub>2</sub> tension is high and pH is between 7.6 and 7.8. Drepanocyte shapes in deer blood vary depending on the hemoglobin types present. Polymerization of hemoglobin in tubular filaments occurs in the blood of some healthy adult Angora goats and some breeds of British sheep. The resultant fusiform or spindle-shaped erythrocytes resemble drepanocytes in deer blood; they have been called *acuminocytes* by some authors. The proportion of fusiform cells in blood of Angora goats varies depending on the individual goat and on in vitro alterations in temperature, pH, and oxygenation.

## 5.3.2.17 Dacryocytes

Dacryocytes are teardrop-shaped with single elongated or pointed extremities. Dacryocytosis is a common feature of myelofibrosis in humans, but dacryocytes are not as commonly recognized in dogs with myelofibrosis. Dacryocytes have also been seen in the blood of dogs and cats with myeloproliferative disorders, dogs with glomerulonephritis, and a dog with hypersplenism. Dacryocyte formation is common in iron-deficient ruminants, including llamas.

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## 5.3.2.18 Nucleated Erythrocytes

Metarubricytes and rubricytes are seldom present in the blood of healthy adult mammals, although low numbers may occur in the blood of some healthy dogs and cats. These nucleated erythrocytes are often seen in blood in association with regenerative anemia (see [Plate 1](#)); however, their presence does not necessarily indicate that a regenerative response is present ([Plate 16](#)). Nucleated erythrocytes are rarely seen in horses with regenerative anemia.

Nucleated erythrocytes may be seen in animals with lead poisoning, during which there is minimal or no anemia, and in animals with nonanemic conditions in which bone marrow is damaged, such as septicemia and endotoxic shock or after administration of some drugs. Low numbers of nucleated erythrocytes are seen in a wide variety of conditions in dogs including cardiovascular disease, trauma, hyperadrenocorticism, and

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various inflammatory conditions. Similarly, metarubricytosis has been reported in cats with hepatic lipidosis, acute trauma, and inflammatory conditions.

When frequent nucleated erythrocyte precursors are present in the blood of an animal with nonregenerative anemia, conditions including myelodysplasia, hematopoietic neoplasia, infiltrative marrow disease, impaired splenic function, and inherited dyserythropoietic disorders should be considered. Erythrocyte nuclei may be lobulated or fragmented in animals with myeloproliferative disorders or in those who have received chemotherapy with drugs that induce nuclear fragmentation.

## 5.3.2.19 Howell-Jolly Bodies (Micronuclei)

The small, spherical nuclear remnants called *Howell-Jolly bodies* (see [Plate 1](#)) form in the bone marrow and are removed by “pitting” action of the spleen. They may be present in low numbers in erythrocytes of healthy cats and horses. Howell-Jolly bodies are often increased in association with regenerative anemia or after splenectomy in various species. They may be increased in animals receiving glucocorticoid therapy and in animals being treated with chemotherapeutic agents that induce nuclear fragmentation, such as vincristine, colchicine, and cytosine arabinoside.

## 5.3.2.20 Heinz Bodies

Heinz bodies are large aggregates of oxidized, precipitated hemoglobin that are attached to the internal surfaces of erythrocyte membranes. In contrast to Howell-Jolly bodies, which stain dark blue, they stain red to pale pink with Romanowsky-type stains ([Plate 5](#)). Heinz bodies appear light blue with reticulocyte stains ([Plate 6](#)). They can also be visualized as dark, refractile inclusions in new methylene blue “wet” preparations ([Fig. 4-7](#)). In contrast to other domestic animal species, healthy cats may have up to 5% Heinz bodies within their erythrocytes. Not only is cat hemoglobin more susceptible to denaturation by endogenous oxidants, but the cat spleen is less efficient in the removal (pitting) of Heinz bodies from erythrocytes than the spleens of other species. An increased number of Heinz bodies may occur with minimal anemia in cats with spontaneous diseases, such as diabetes mellitus (especially when ketoacidosis is present), hyperthyroidism, and lymphoma. Small Heinz bodies are seen in other species after splenectomy.

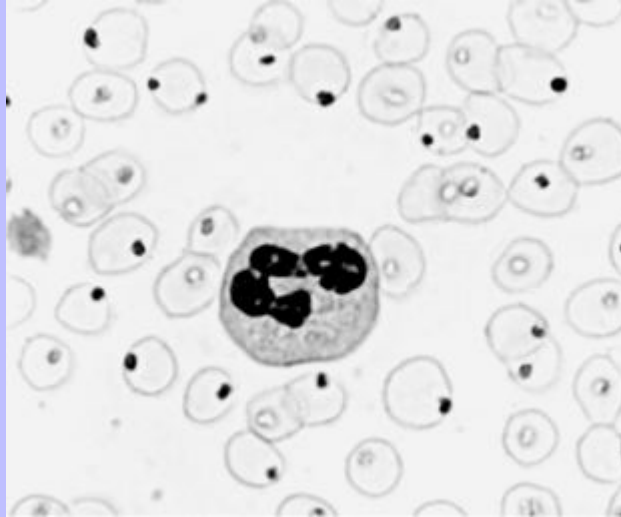
Dietary causes of Heinz body hemolytic anemia include consumption of onions by small and large animals, consumption of kale and other *Brassica* species by ruminants, consumption of lush winter rye by cattle, and consumption of red maple leaves by horses. Heinz bodies have been detected in erythrocytes from selenium-deficient Florida cattle grazing on St. Augustine grass (*Stenotaphrum secundatum*) and in postparturient New Zealand cattle grazing primarily on perennial ryegrass. Copper toxicity results in Heinz body formation in sheep and goats. Heinz body formation has been reported in some dogs ingesting zinc-containing objects (e.g., U.S. pennies minted after 1982). Naphthalene ingestion may have caused Heinz body formation in a dog. Heinz body hemolytic anemia has occurred after administration of a variety of drugs, including acetaminophen and methylene blue in cats and dogs, methionine and phenazopyridine in cats, menadione (vitamin K<sub>3</sub>) in dogs, and phenothiazine in horses. Ingestion of crude oil has resulted in Heinz

body hemolytic anemia in marine birds.

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Fig. 4-7



The new methylene blue wet-mount staining procedure was used to demonstrate Heinz bodies in cat erythrocytes. A neutrophil is also present.

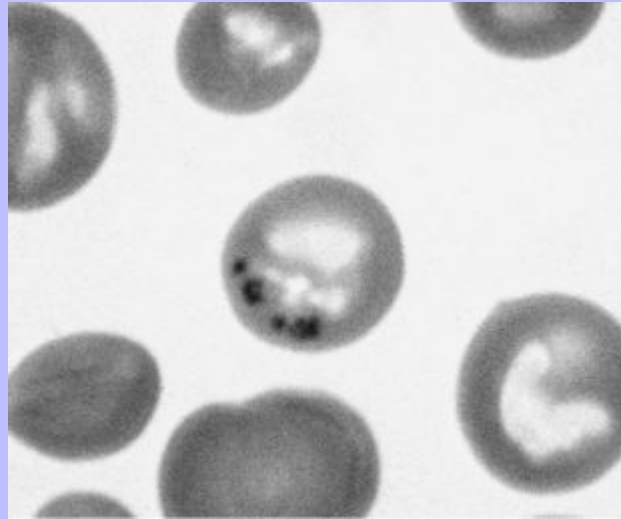
### 5.3.2.21 Basophilic Stippling

Reticulocytes usually stain as polychromatophilic erythrocytes with Romanowsky-type blood stains because of the presence of dispersed ribosomes and polyribosomes, but sometimes the ribosomes and polyribosomes aggregate together, forming blue-staining punctate inclusions referred to as *basophilic stippling*. These aggregates are similar to those produced when reticulocyte stains are used, but they form during the process of cell drying before staining with Romanowsky-type blood stains. Diffuse basophilic stippling commonly occurs in regenerative anemia in ruminants (see [Plate 4](#)) and occasionally occurs in regenerative anemia in other species. Basophilic stippling may be prominent in any species with lead poisoning.

### 5.3.2.22 Siderotic Inclusions

Siderotic inclusions often appear as focal basophilic stippling on routinely stained blood films ([Fig. 4-8](#)), and erythrocytes containing these iron-positive inclusions are called *siderocytes*. A Prussian blue staining procedure is used to verify the presence of iron-positive material. Siderocytes are rare or absent in the blood of healthy animals. They may occur with lead poisoning, hemolytic anemia, dyserythropoiesis, myeloproliferative diseases, chloramphenicol therapy, hydroxyzine therapy (in dogs), and experimental pyridoxine deficiency (in pigs). Siderotic inclusions have been recognized in a dog with zinc toxicity, but it is unclear whether this was a result of the zinc toxicity per se or was associated with the accompanying hemolytic anemia.

Fig. 4-8



A siderocyte with focal basophilic stippling in Wright-Giemsa–stained blood from a dog treated with chloramphenicol. Erythrocytes containing focal basophilic stippling were classified as siderocytes on the basis of positive Prussian blue staining. (From Harvey JW, Wolfsheimer KJ, Simpson CF, et al: Pathologic sideroblasts and siderocytes associated with chloramphenicol therapy in a dog. *Vet Clin Pathol* 1985;14:36.)

### 5.3.2.23 Infectious Agents

A number of infectious organisms are commonly detected in or on erythrocytes. *Babesia* species (Fig. 4-9), *Theileria* species, and *Cytauxzoon felis* (Fig. 4-10) are intracellular protozoa. *Anaplasma* species (see Plate 4) are intracellular rickettsial organisms found in ruminants. Hemotrophic mycoplasmas (formerly in the *Haemobartonella* and *Eperythrozoon* genera) are epicellular organisms that attach to the outside of erythrocytes (Figs. 4-11 and 4-12; Plate 7). Distemper inclusions may be seen in dog erythrocytes (Plate 8) during viremia. *Bartonella* species are rod-shaped bacteria that occur within erythrocytes, but they are rarely appreciated on blood films of animals with bacteremia. It is important to differentiate these various infectious agents from stain artifacts, Howell-Jolly bodies, and platelets overlying erythrocytes.

### 5.3.3 Reticulocyte Counts

Manual methods used in performing reticulocyte staining and counting are described in Chapter 2. In healthy cats, as well as cats with regenerative anemia, the number of punctate reticulocytes is much greater than that seen in other species. This apparently occurs because the maturation (loss of ribosomes) of reticulocytes in cats is slower than that in other species. Consequently, reticulocytes in cats are classified as aggregate (if coarse clumping is observed) or punctate (if small individual inclusions are present). Percentages of both types should be reported separately. According to composite results from several authors, healthy cats generally have from 0% to 0.5% aggregate and 1% to 10% punctate reticulocytes when counts are done manually. Higher punctate counts (2% to 17%) have been reported when sensitive flow cytometry was used. The percentage of aggregate

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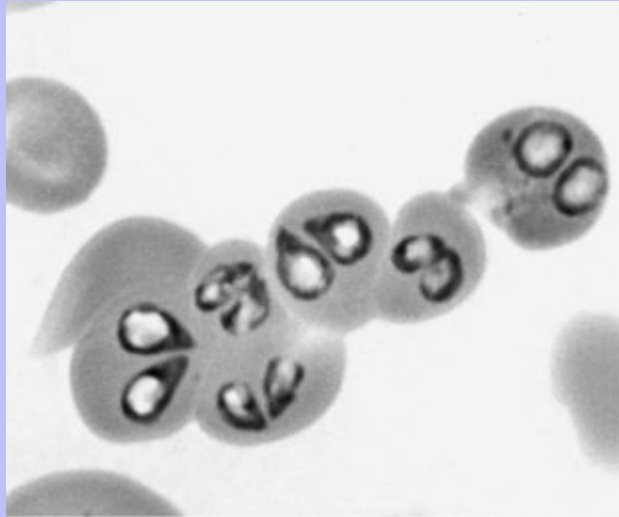
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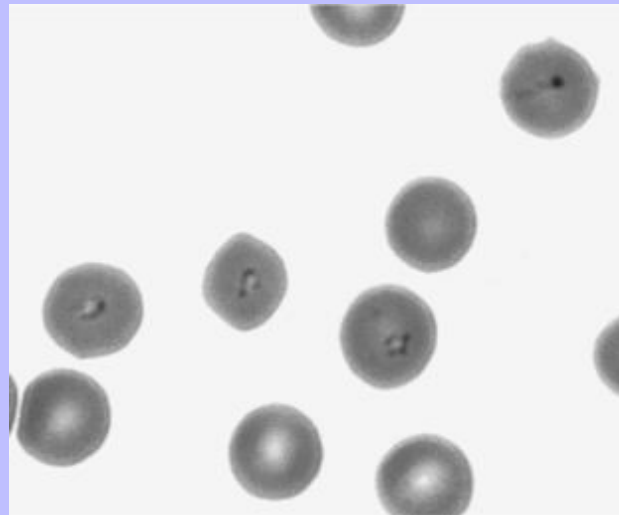
reticulocytes correlates directly with the percentage of polychromatophilic erythrocytes observed on blood films stained with Wright-Giemsa stain. Aggregate reticulocytes in the circulation mature to punctate reticulocytes in a day or less. A week or more is required for maturation (total disappearance of ribosomes) of punctate reticulocytes in cat blood. In contrast to those of the cat, most reticulocytes in other species are of the aggregate type. Consequently, no attempt is made to differentiate stages of reticulocytes in species other than cats.

Fig. 4-9



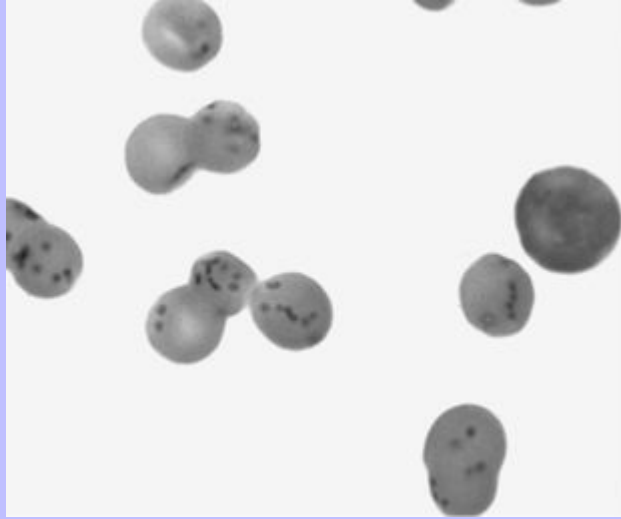
Dog erythrocytes containing *Babesia canis* organisms. (Wright-Giemsa stain.)

Fig. 4-10



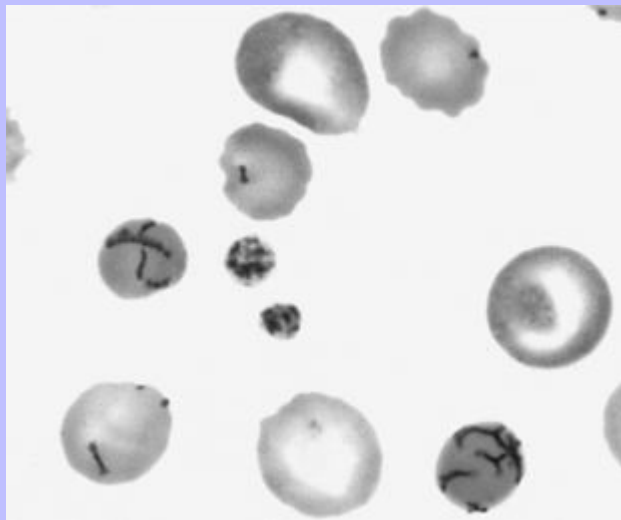
Cat erythrocytes containing *Cytauxzoon felis* organisms. (Wright-Giemsa stain.)

Fig. 4-11



Cat erythrocytes parasitized by *Mycoplasma haemofelis* (*Haemobartonella felis*) organisms. A nucleated erythrocyte and neutrophil are present. (Wright-Giemsa stain.)

Fig. 4-12



Dog erythrocytes parasitized by *Mycoplasma haemocanis* (*Haemobartonella canis*) organisms. (Wright-Giemsa stain.)

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Raw (uncorrected) manual reticulocyte counts can be misleading when moderate to severe anemia is present because reticulocytes are quantified as a percentage of total erythrocytes (reticulocytes plus mature erythrocytes)

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counted. The raw reticulocyte count (percentage) would be higher in an anemic animal (with a lower number of mature erythrocytes) than it would be in a healthy animal (with a higher number of mature erythrocytes), even if the actual number of reticulocytes per microliter in the circulation were the same in each animal. Consequently, reticulocyte counts should be corrected for the degree of anemia by using the HCT, or an absolute reticulocyte count should be calculated by using the total RBC count. The reticulocyte count is corrected by dividing the patient's HCT by the mean normal HCT for the species and multiplying this value times the raw reticulocyte count to obtain a corrected reticulocyte count.

$$\text{Corrected reticulocyte count (\%)} = \frac{\text{Patient's HCT}}{\text{Mean Normal HCT}} \times \text{Raw reticulocyte count (\%)}$$

For example, if the raw reticulocyte count was determined to be 6% in a dog with an HCT of 9%, then the corrected reticulocyte count would be 9 divided by 45 (mean normal HCT for dogs) times 6% = 1.2%. The corrected reticulocyte count is used to determine whether reticulocytes are truly increased in blood. Although the raw reticulocyte count (6%) suggested the reticulocyte numbers were increased substantially in blood, the corrected reticulocyte count demonstrated that little if any increase in reticulocyte numbers was present in blood.

If the total RBC count is known, an absolute reticulocyte count (reticulocytes per microliter) can be determined. This is done by multiplying the percentage of reticulocytes counted (expressed as a fraction) times the total RBC count.

$$\begin{aligned} \text{Absolute reticulocyte count (/}\mu\text{L)} \\ = \text{RBC count (/}\mu\text{L)} \times \text{Raw reticulocyte count (fraction)} \end{aligned}$$

Absolute reticulocyte counts above 80,000/ $\mu\text{L}$  are considered to be increased in dogs. Healthy cats have between 0 and 30,000/ $\mu\text{L}$  aggregate reticulocytes, but punctate reticulocyte counts may be as high as 500,000/ $\mu\text{L}$ . It should be noted that the calculations of absolute reticulocyte counts and corrected reticulocyte counts are independent calculations, with each one using the original raw reticulocyte count.

Absolute reticulocyte counts can also be determined directly by flow cytometry in some automated hematology analyzers. It is critical that these automated counts be validated by comparing results with manual reticulocyte counts. It is especially important to determine whether all reticulocytes or only aggregate reticulocytes are counted in cat blood.

The corrected reticulocyte response to blood loss anemia in the cat is shown in [Figure 4-13](#). As in other species, about 4 days are required for maximal aggregate reticulocyte response to anemia because of the time required for the production of aggregate reticulocytes from progenitor cells. The maximal punctate response occurs considerably later, primarily because of the long time required for punctate reticulocytes to mature to erythrocytes. As can be seen in [Figure 4-13](#), punctate reticulocyte release from bone marrow continues after the HCT begins to increase and aggregate reticulocyte release has ceased. Consequently, cats with mild anemia may have increased punctate reticulocyte counts and normal aggregate reticulocyte counts.

Lower HCTs typically result in higher plasma erythropoietin (EPO) concentrations, which result in higher absolute blood reticulocyte counts (except in horses), if the marrow is able to respond appropriately. Absolute reticulocyte counts are generally higher in response to hemolytic anemia than they are in response to hemorrhage, presumably because the plasma iron concentration is high in animals with hemolytic anemia and normal or low in animals after hemorrhage. Consequently, the presence of marked reticulocytosis indicates the likelihood that increased erythrocyte destruction is the cause of the anemia. When the degree of anemia is severe, basophilic macroreticulocytes, or so-called stress reticulocytes, may be released into blood. It is proposed that one less mitotic division occurs during production and that immature reticulocytes, nearly twice the normal size, are released. Although a portion of these macroreticulocytes are apparently rapidly removed from the circulation,

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it appears from studies in cats that some can mature into large (macrocytic) erythrocytes with relatively normal life spans.

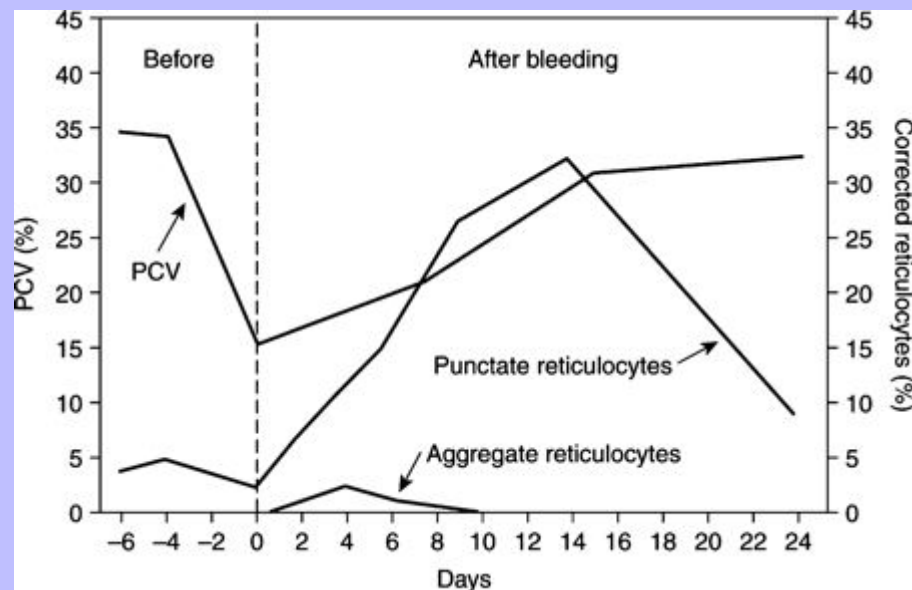
### 5.3.4 Erythrocyte Indices

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Determination of erythrocyte indices can assist in the differential diagnosis of anemia. Of the parameters routinely determined or calculated, the MCV is the most useful.

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Fig. 4-13



Reticulocyte response after controlled bleeding in cats. Reticulocyte counts have been corrected by using hematocrit values. PCV, Packed cell volume. The graph includes data from Alsaker RD, Laber J, Stevens JB, et al: A comparison of polychromasia and reticulocyte counts in assessing erythrocyte regenerative response in the cat. *J Am Vet Med Assoc* 1977;170:39.

#### 5.3.4.1 Mean Cell Volume

The MCV represents the average volume of a single erythrocyte in femtoliters ( $10^{-15}$  liters). The MCV is determined most accurately by direct measurement with electronic cell counters. It can be determined indirectly by dividing the HCT (as a percentage) by the RBC count (in millions of cells per microliter) and multiplying by 10, but this calculated value is less accurate because two separate measurements are required. The MCV varies greatly by species. Mammals have smaller erythrocytes than birds, reptiles, or amphibians. Species with larger erythrocytes have lower RBC counts, resulting in similar HCTs in mammals and birds. MCVs can vary with age, with higher MCVs reported in older horses and cattle. Slight increases in MCVs (about 1 fL) occur with high-speed exercise in horses.

High MCV values (macrocytosis) are usually associated with regenerative anemias because the volumes of individual reticulocytes (especially stress reticulocytes) are larger than the volumes of mature erythrocytes.

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However, it is important to remember that many macrocytic cells need to be present to increase the MCV above the normal reference interval. Some dogs with nonregenerative immune-mediated anemia and/or myelofibrosis also have macrocytosis. High MCVs may occur in animals with myeloproliferative disorders. Macrocytosis is often seen in feline leukemia virus (FeLV)-positive cats with nonregenerative anemias. Folate deficiency has been reported as a cause of macrocytic nonregenerative anemia in a cat. Macrocytosis (without anemia or reticulocytosis) occurs in some apparently healthy poodles. Dogs with hereditary stomatocytosis may have high MCVs, with normal or only slightly increased reticulocyte counts. Some cats with hyperthyroidism have slightly increased MCVs with normal or increased HCT. Macrocytic anemia has been reported in Hereford calves with congenital dyserythropoiesis; many nucleated erythrocytes are present in blood, but reticulocyte counts are only slightly increased in these calves.

High MCVs may occur as an artifact caused by agglutination of erythrocytes, as may occur in immune-mediated disorders or after heparin administration to horses. MCVs may also be spuriously increased in cats and dogs with persistent hyponatremia because the cells can swell in vitro when blood is diluted with counting fluid before sizing in an electronic cell counter. Finally, MCVs increase with prolonged storage of blood samples.

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Macrocytosis is more likely to occur in response to hemolytic anemia than in response to hemorrhage, at least in part because the serum iron concentration is increased in animals with hemolytic anemia. Although iron does not stimulate erythropoiesis, decreased iron availability may limit the erythropoietic response after hemorrhage. Reticulocytes, especially those produced in response to severe anemia (stress reticulocytes), are larger than mature erythrocytes. A week or more is required before macrocytosis occurs in response to hemolytic anemia. Although the bone marrow normally contains some reticulocytes undergoing maturation, most reticulocytes released from the bone marrow in response to anemia must be formed de novo. Four or 5 days are required for a peak reticulocyte response to occur, and then the newly produced, larger cells must comprise a high enough percentage of the total erythrocytes present to increase the MCV above the reference interval. Although there is a reduction in size as reticulocytes mature into erythrocytes, larger than normal reticulocytes produce larger than normal erythrocytes.

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The erythrocytes produced in the fetus are larger than those produced in adults. There is a gradual decrease in MCV during fetal development. The MCV is within adult reference intervals in horses and cattle at birth. The MCV is above adult reference intervals in dogs and cats at birth, and it declines as the larger erythrocytes formed in the fetus are replaced by smaller erythrocytes produced after birth.

Microcytic (low MCV) anemias usually indicate the presence of iron deficiency. Microcytic iron deficiency anemia in adult animals is almost always due to chronic hemorrhage. Depending on the initial MCV and magnitude of ongoing blood loss, one or more months are required before the MCV decreases below the reference interval. Body iron stores must be depleted and then the microcytes formed must comprise a high enough percentage of the total erythrocytes present to decrease the MCV below the reference interval. Microcytic anemia rarely occurs as a result of dietary iron deficiency in adult animals. However, iron deficiency without blood loss is common in nursing animals because milk is low in iron and there is increased demand for iron in these rapidly growing animals. Although microcytes are often formed in nursing animals, the MCV may not be reduced in neonatal dogs and cats because of the persistence of macrocytes formed before birth. Erythrocytes may become microcytic in dogs with primary erythrocytosis and in dogs given long-term recombinant canine EPO therapy, even though body iron stores have not been depleted. Microcytosis apparently occurs because iron delivery to the developing erythroid cells is not sufficient to fully support the accelerated erythropoiesis accompanying recombinant canine EPO administration.

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Copper is needed for optimal iron absorption and release from body iron stores. Consequently, prolonged copper deficiency results in microcytic anemia in some species. Pyridoxine is required for the first step in heme synthesis. Although natural cases of pyridoxine deficiency have not been documented in domestic animals, microcytic anemias with high serum iron values have been produced experimentally in dogs, cats, and pigs with dietary pyridoxine deficiency.

The MCV may be slightly decreased in association with the anemia of inflammatory disease, but the MCV is at the low end of the reference interval in most cases. Microcytosis is common in dogs with portosystemic shunts. In these cases, the MCV is seldom more than 7 fL below the reference interval, and the HCT is within the reference interval or only slightly decreased. Some cats with portosystemic shunts and hepatic lipidosis exhibit slight microcytosis. Drugs or chemicals that block heme synthesis, such as chloramphenicol and lead, have the potential to cause formation of microcytic erythrocytes that may contain siderotic inclusions. Microcytic anemia may also occur in myeloproliferative disorders exhibiting iron accumulation in erythroid cells. A nonregenerative microcytic anemia with many circulating nucleated erythrocytes has been reported in related English springer spaniels with dyserythropoiesis, polymyopathy, and heart disease. Microcytosis has been described in a cross-bred dog with persistent elliptocytosis, resulting from a lack of erythrocyte membrane protein band 4.1. Although the animal was not anemic, the reticulocyte count was about twice the normal value in response to a shortened erythrocyte life span. Some Japanese dog breeds (Akita and Shiba) normally have MCV values below the reference intervals established for other breeds of dogs, but these dogs are not anemic.

Spurious microcytosis may occur when platelets are included with erythrocytes in MCV calculations in animals with severe anemia or marked thrombocytosis. MCVs may also be spuriously decreased in dogs with persistent hyponatremia because the erythrocytes shrink when blood is diluted in vitro with counting fluid before sizing in an electronic cell counter.

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## 5.3.4.2

### Mean Cell Hemoglobin Concentration

The mean cell hemoglobin concentration (MCHC) represents the average hemoglobin concentration within erythrocytes. It is calculated by dividing the hemoglobin value (in grams per deciliter) by the HCT (as a percentage) and multiplying by 100. The MCHC is expressed as g/dL of erythrocytes. (Note: Hemoglobin values in blood are expressed as g/dL of whole blood.) Reference intervals established by using HCT values determined by electronic cell counters tend to be slightly higher than those determined by using HCT values measured by centrifugation because of the presence of small amounts of trapped plasma in centrifuged samples.

High MCHC values are artifacts. They may result from in vivo or in vitro hemolysis, lipemia, or the presence of Heinz bodies. In the case of hemolysis, some hemoglobin is free in plasma, but the formula used to calculate the MCHC assumes that all measured hemoglobin is contained within erythrocytes. Lipemia and Heinz bodies cause turbidity in the spectrophotometrical assay for hemoglobin, resulting in erroneously elevated hemoglobin values. A high MCHC may also occur if agglutination of erythrocytes occurs when assays are performed in an electronic cell counter, as may occur with cold-acting autoantibodies or after heparin therapy in some horses. Large erythrocyte aggregates are too large to be considered erythrocytes; consequently, cell counters are programmed to exclude them from erythrocyte measurements. This results in erroneously low HCT values, and consequently, in erroneously high MCHC values. Agglutination should not interfere with HCT values determined by centrifugation, as long as blood samples are well mixed before microhematocrit tubes are filled. The MCHC is not falsely elevated by these factors if it is determined by density measurement (e.g., VetAutoread Hematology System [IDEXX Laboratories, Inc, Westbrook, ME]).

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MCHC values may be decreased in animals with regenerative anemia, especially those with high percentages of stress reticulocytes. Hemoglobin synthesis is not complete until late in reticulocyte maturation. Consequently, hemoglobin synthesis is less complete in stress reticulocytes because these cells are released from the bone marrow earlier than normally occurs.

Low MCHC values may also occur in animals with chronic iron deficiency anemia. When determined by electronic cell counters, the MCHC is usually normal in animals with slight microcytosis, but it is usually low when MCV is markedly reduced. The MCHC is low in iron-deficient animals because of inadequate iron to support synthesis of normal amounts of hemoglobin. Low MCHC values occur in dogs with hereditary stomatocytosis because the increased intracellular water, which occurs in this condition, dilutes the hemoglobin concentration within cells. MCHCs may be spuriously decreased in cats and dogs with persistent hypernatremia because the cells can swell when blood is diluted with counting fluid before electronic cell counter analysis.

5.3.4.3

## Mean Cell Hemoglobin

The mean cell hemoglobin is calculated by dividing the hemoglobin value (in grams per deciliter) by the RBC count (in millions per microliter) and multiplying by 10. The mean cell hemoglobin provides no added value because it depends on the MCV and MCHC. It usually correlates directly with the MCV, except in animals with macrocytic hypochromic erythrocytes.

5.3.4.4

## Red Cell Distribution Width

The red cell distribution width (RDW) is an electronic measure of anisocytosis or erythrocyte volume heterogeneity. A histogram plot of the volume of individual erythrocytes reveals a plot that approximates a Gaussian distribution. Consequently, one can calculate the degree of size variation by determining the standard deviation (SD) of erythrocyte volumes. However, the SD depends on the size of the cells, as well as the degree of size variation around the MCV. The coefficient of variation of erythrocyte volume is calculated by dividing the SD by the MCV and then multiplying by 100 to provide a measure of size variation that does not depend on how large the cells are. In short, the RDW is the SD of erythrocyte volumes expressed as a percentage of the mean erythrocyte volume.

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Reference values vary depending on the instrument used to measure the RDW. Cattle and horses normally have higher RDW values than cats and dogs. One need only refer to the upper limit of a reference interval when examining data from a patient because there is no pathologic state in which erythrocytes have greater volume homogeneity (lower RDW) than normal.

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Examination of the RDW has not been extensively used in veterinary medicine. The RDW is expected to be increased in cases in which the degree of anisocytosis (as estimated on the stained blood film) is increased. It is often increased in regenerative anemias because reticulocytes and young erythrocytes are larger than mature erythrocytes. As with the MCV, the number of large erythrocytes in blood must reach a certain level before the RDW of a given patient exceeds the reference interval. In some animals, the RDW will be increased before the MCV exceeds the reference interval. As an animal responds to anemia and young erythrocytes become the predominant population, the RDW will begin to decline and may return to the reference interval, even though the MCV is still high.

The RDW is also expected to increase in cases of iron deficiency anemia in which smaller than normal erythrocytes are produced. As in regenerative anemia, the increase is most likely to be seen during the phase

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of disease when there are a significant number of normally and abnormally sized erythrocytes present simultaneously. In severe chronic iron deficiency anemia, the RDW might decrease toward normal once the whole population of erythrocytes is small. The RDW may increase again after iron therapy, as normally sized erythrocytes are produced.

Other causes of increased RDW include conditions in which substantial fragmentation of erythrocytes is occurring and after transfusion of blood from a donor animal in which the MCV is substantially different from that of the recipient. The RDW is also increased in dogs with hereditary stomatocytosis. Animals with nonregenerative anemias will have normal RDW values unless significant dyserythropoiesis is present. Spuriously increased RDW values may occur when agglutination is present or when platelets are included with erythrocytes in cell volume distribution calculations of animals with severe anemia.

## 5.3.5 Erythrocyte Volume Histograms and Erythrocyte Cytograms

Although quite useful when abnormal, MCV and MCHC values are relatively insensitive in identifying the presence of erythrocytes with abnormal volumes or hemoglobin concentrations. Many microcytic or macrocytic erythrocytes are required to move the MCV below or above the reference interval, respectively, and many hypochromic erythrocytes are needed to move the MCHC below the reference interval. In addition to counting cells, electronic cell counters can determine and plot the volume of individual erythrocytes, and examination of these erythrocyte volume histograms can reveal the presence of increased numbers of microcytes or macrocytes, even when the MCV is within the reference interval. Some electronic cell counters such as the Advia 120 (Bayer Diagnostics, Tarrytown, NY) can also determine the hemoglobin concentration of individual erythrocytes from the deflection of light that occurs when a laser beam strikes individual cells. Inspection of hemoglobin concentration histograms can reveal the presence of increased numbers of hypochromic erythrocytes, even when the MCHC has not decreased below the reference interval. Individual erythrocytes can be further characterized by creating a cytogram in which the erythrocyte volumes of individual cells are plotted against their respective hemoglobin concentrations.

## 5.3.6 Direct Antiglobulin Test

A direct antiglobulin (Coombs') test is done when autoagglutination is absent, but immune-mediated hemolytic anemia is still suspected. Species-specific antisera against IgG, IgM, and the third component of complement (C<sub>3</sub>) are used to detect the presence of one or more of these factors on the surface of erythrocytes. This test is discussed in greater detail in [Chapter 7](#).

## 5.3.7 Serum Iron Assays

Serum iron concentration is increased in animals with hemolytic anemias and in dogs and horses after the administration of glucocorticoid steroids. Serum iron is decreased after glucocorticoid administration to cattle. It is low in iron deficiency, the anemia of inflammatory disease, and some dogs with portosystemic shunts. The total iron-binding capacity (TIBC) of serum is a measure of serum transferrin concentration because insignificant amounts of circulating iron are bound to other proteins. Serum TIBC is low-normal or decreased in the anemia of inflammatory disease and increased in some species with iron deficiency. The TIBC is normal in dogs with chronic iron deficiency anemia.

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These disorders can usually be differentiated by examination of bone marrow for stainable iron, which is minimal or absent in iron deficiency and normal or high in the anemia of inflammatory disease. Stainable iron is

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not present in the bone marrow of healthy cats; consequently, a lack of stainable iron does not suggest iron deficiency in this species.

Serum ferritin concentration correlates with tissue iron stores in domestic animals, including cats. Consequently, serum ferritin concentration can help differentiate true iron deficiency (serum ferritin is low) from the anemia of inflammatory disease (serum ferritin is normal or high), but commercial assay kits for ferritin are only available for humans. Serum ferritin is an acute-phase protein; consequently, increased values are expected in inflammatory conditions. Increased serum ferritin values also occur in chronic hemolytic anemias (e.g., pyruvate kinase deficiency) and in canine malignant histiocytosis. Serum ferritin is transiently increased in horses after moderate to severe exercise.

### 5.3.8 Methemoglobin Determination

Hemoglobin is a protein consisting of four polypeptide globin chains, each of which contains a heme prosthetic group within a hydrophobic pocket. Heme is composed of a tetrapyrrole with a central iron molecule that must be maintained in the ferrous (+2) state to reversibly bind O<sub>2</sub>. Methemoglobin differs from hemoglobin only in that the iron moiety of heme groups has been oxidized to the ferric (+3) state and is no longer able to bind O<sub>2</sub>. Methemoglobinemia refers to methemoglobin content in blood greater than 1.5% of total hemoglobin. Clinical signs associated with methemoglobinemia are the result of hypoxia because methemoglobin cannot bind O<sub>2</sub>. Both low blood O<sub>2</sub> tension and methemoglobinemia can result in cyanotic appearing mucous membranes and dark-colored blood samples. Hypoxemia is documented by measuring a low Po<sub>2</sub> in an arterial blood (Pao<sub>2</sub>) sample. Methemoglobinemia is suspected when arterial blood with normal or increased Pao<sub>2</sub> is dark-colored. Methemoglobin is quantified spectrophotometrically, but a spot test can be used to determine whether clinically significant levels of methemoglobin are present. One drop of blood from the patient is placed on a piece of absorbent white paper, and a drop of normal control blood is placed next to it. If the methemoglobin content is 10% or greater, the patient's blood should have noticeably brown coloration. Methemoglobinemia results from either increased production of methemoglobin by oxidants or decreased reduction of methemoglobin, resulting from a deficiency in the erythrocyte methemoglobin reductase enzyme.

#### 5.3.8.1 Toxic Methemoglobinemia

Significant methemoglobinemia has been associated with clinical cases of benzocaine, acetaminophen, and phenazopyridine toxicities in cats and dogs; copper toxicity in sheep and goats; and red maple toxicity in horses. These oxidants can also produce Heinz body hemolytic anemias. Nitrite produces methemoglobinemia without Heinz body formation or development of anemia. Methemoglobinemia occurs in ruminants eating nitrate-accumulating plants, especially when the plants have been fertilized with nitrogenous compounds. Nitrate is relatively nontoxic, but it is reduced to nitrite by ruminal microorganisms.

#### 5.3.8.2 Methemoglobin Reductase Deficiency

Persistent methemoglobinemia resulting from erythrocyte methemoglobin reductase (cytochrome-*b*<sub>5</sub> reductase) deficiency has been recognized in many breeds of dogs and in domestic short-haired cats. Methemoglobin content is higher in cats (44% to 52%) than in dogs (13% to 41%) with this deficiency because of lower enzyme activity in deficient cats compared with deficient dogs. Flavin adenine dinucleotide is a cofactor for the methemoglobin reductase enzyme, and persistent methemoglobinemia (26% to 48%) has also been detected in a mustang and a Kentucky mountain saddle horse with methemoglobin reductase

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deficiency associated with erythrocyte flavin adenine dinucleotide deficiency. In contrast to	67
methemoglobinemia produced by oxidant drugs and compounds, animals with methemoglobin reductase	68
deficiency usually exhibit no clinical signs of illness. The diagnosis of this deficiency is made by measuring enzyme activity within erythrocytes.	

## 5.3.9 Erythropoietin Assay

EPO is a glycoprotein hormone that stimulates erythropoiesis in a number of ways. Radioimmunoassays can be used to measure EPO, but commercial tests developed for human assays may not always cross-react sufficiently for use with other species. Consequently, individual radioimmunoassays require validation for each species to be tested before they can be used clinically. Serum EPO is increased in response to various anemias, except the anemia of chronic renal disease, in which EPO production is decreased. Serum EPO concentration appears to be regulated not only by the rate of renal production but also by the rate of utilization by erythroid cells. At any given hemoglobin level, the serum EPO concentration is likely to be highest in disorders with low marrow erythroid activity (e.g., erythroid aplasia). The EPO assay has had limited use in veterinary medicine. EPO has been assayed in serum to assist in differentiating primary erythrocytosis (in which EPO values should be normal or low) from secondary erythrocytosis (in which EPO values should be high). Unfortunately, there is considerable overlap in values among patients with primary and secondary erythrocytosis, limiting the diagnostic value of the EPO assay.

## 5.4 DIFFERENTIAL DIAGNOSIS OF ANEMIA

True or absolute anemia is defined as a decrease in erythrocyte mass within the body. HCT, hemoglobin, and RBC count values are usually below their reference intervals; however, the anemia can sometimes be masked by concomitant dehydration. Low erythrocyte values may also be present in blood when the total body erythrocyte mass is normal (relative anemia). This can result from overhydration, resulting in erythrocyte dilution, and from splenic sequestration of erythrocytes, as occurs with splenic relaxation during anesthesia, heparin-induced erythrocyte agglutination in horses, and various causes of splenomegaly.

Anemia is a problem, not a diagnosis. Anemia is classified in various ways to assist in determining its specific cause so that effective therapy can be provided. In addition to history, presenting complaints, and laboratory findings, results of other test procedures (e.g., diagnostic imaging) are important in reaching a final diagnosis.

Anemia may occur after blood loss, increased erythrocyte destruction, or decreased erythrocyte production. Factors that can be useful in categorizing anemia into these broad causes (and often into more specific causes) include reticulocyte counts, erythrocyte indices, erythrocyte morphology observed on stained blood films, the appearance of the plasma, plasma protein concentration, serum iron measurements, serum bilirubin determination, direct antiglobulin test, and bone marrow evaluation.

Anemia may also develop when the expansion of the vascular space occurs more rapidly than the expansion of the total body erythrocyte mass. This hemodilution contributes to the anemia of the neonate (to be discussed later) and to a mild anemia that develops during pregnancy in most domestic animals, the horse being an exception.

### 5.4.1 Regenerative Versus Nonregenerative Anemia

The most useful approach in the classification of anemia is to determine whether evidence of a bone marrow response to the anemia is present in blood. For all species except the horse, this involves determining whether absolute reticulocyte numbers are increased in blood. Horses rarely release reticulocytes from the bone marrow,

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even when increased production of erythrocytes occurs. MCV and/or RDW values are increased in some horses responding to anemia, but others recover from anemia without these values exceeding reference intervals. Consequently, bone marrow evaluation is often needed to determine whether an appropriate response to anemia is present in a horse. Myeloid to erythroid (M/E) ratios below 0.5 and bone marrow reticulocyte counts above 5% suggest a regenerative response to anemia.

Increased polychromasia is usually present in regenerative anemias because many reticulocytes stain bluish red with routine blood stains. Cats with mild anemias may not release aggregate reticulocytes from the marrow but will release punctate reticulocytes. Because punctate reticulocytes do not contain sufficient numbers of

ribosomes within them to impart a bluish color to the cytoplasm, mild regenerative anemias in cats may lack polychromasia on stained blood films. Increased anisocytosis is often present in regenerative anemias because of the presence of large immature erythrocytes, although anisocytosis may be marked in some nonregenerative anemias as well.

Some nucleated erythrocytes (rubricytes and metarubricytes) are often seen on blood films in association with regenerative anemia (except in horses); however, nucleated erythrocytes may also be present in anemic and nonanemic disorders with minimal or no reticulocytosis. Therefore the presence of nucleated erythrocytes in blood is a much less reliable indicator of a regenerative response to anemia than is an increased reticulocyte count.

Howell-Jolly bodies are often present within erythrocytes in regenerative anemias, but they also occur in healthy cats and horses and in splenectomized animals of other species. Basophilic stippling occurs in regenerative anemias in ruminants, but rarely in other species. Basophilic stippling can also occur in erythrocytes of any species with lead toxicity, regardless of whether anemia is present.

The presence of compensatory reticulocytosis indicates that the anemia has resulted from either blood loss or increased erythrocyte destruction. Several factors should be kept in mind when the magnitude of a reticulocyte response is interpreted. In regenerative anemias, animals with lower HCTs should have higher absolute reticulocyte counts. Severe anemia evokes a greater stimulus for increased erythrocyte production than mild anemia. Also, in response to severe anemia, reticulocytes can be released from the marrow earlier in their development than normally occurs. These large “stress” reticulocytes apparently remain in the circulation longer than other reticulocytes before maturation is complete. Factors have been used in an attempt to correct for this longer reticulocyte circulation time in humans, and some veterinary authors have empirically applied the same factors to anemic dogs to calculate what has been called the *reticulocyte index*. This approach has not been validated in dogs, and its use in humans has recently been questioned.

Hemolytic anemia usually elicits a more dramatic regenerative response than hemorrhagic anemia, at least in part because of greater availability of iron. There are also species differences in the ability to increase erythrocyte production. Dogs respond more dramatically with higher reticulocyte counts, more polychromasia, and more rapid return of HCT toward normal than do cats in similar disease situations. The return of the HCT to normal is slower in cattle than in cats and slower yet in horses.

Anemias with no increase in or low numbers of reticulocytes are classified as nonregenerative and poorly regenerative, respectively. The lack of a reticulocyte response in nonequine species generally indicates that the anemia results from insufficient erythrocyte production in the marrow. A minimal reticulocyte response may be present if the anemia is documented shortly after acute hemorrhage or hemolysis because about 4 days are required for a substantial reticulocyte response to occur.

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## 5.4.2 Classification of Anemia with Erythrocyte Indices

An anemia can also be classified by using the MCV and MCHC values to assist in determining the cause of the anemia. The terms used for size are *macrocytic* (increased MCV), *normocytic* (normal MCV), and *microcytic* (decreased MCV). Terms used to describe MCHC values are *normochromic* (normal MCHC) and *hypochromic* (decreased MCHC). Anemias are not classified as hyperchromic because high MCHC values are artifacts. A comparison of erythrocyte indices and causes of anemia is provided in [Box 4-2](#).

## 5.5 HEMOLYTIC ANEMIAS

Hemolytic anemias occur as a result of increased erythrocyte destruction within the body. Causes of hemolytic anemia in animals are listed in [Box 4-3](#). Erythrocytes may be lysed within the circulation (intravascular hemolysis), but more frequently, they are lysed after phagocytosis by cells of the mononuclear phagocyte system (extravascular hemolysis).

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Hemolytic anemias are generally regenerative, if sufficient time has elapsed for a bone marrow response to the anemia. They are often macrocytic hypochromic or macrocytic normochromic, but may be normocytic normochromic, if sufficient time has not elapsed for bone marrow release of a significant number of large reticulocytes. Macrocytic hypochromic erythrocytes may also occur in hereditary stomatocytosis in dogs as a result of membrane abnormalities and erythrocyte swelling. An example of a hemolytic anemia that is consistently nonregenerative and normocytic normochromic is cytauxzoonosis in cats. Animals die before there is time for a regenerative response to the anemia to occur. Increased erythrocyte phagocytosis occurs in animals with malignant histiocytosis and in animals with hemophagocytic syndrome, but the anemia may not be regenerative if histiocyte proliferation within the marrow or the associated release of inflammatory mediators interferes with normal erythropoiesis.

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### 5.5.1 BOX 4-2 Comparison of Classification of Anemias by Erythrocyte Indices and Etiology

#### 5.5.1.1 Normocytic Normochromic

1. Acute hemolysis before sufficient time has elapsed for significant reticulocyte response
2. Acute hemorrhage before sufficient time has elapsed for significant reticulocyte response or mild hemorrhage that does not stimulate substantial reticulocyte response
3. Early iron deficiency before microcytes predominate
4. Chronic inflammation and neoplasia (sometimes slightly microcytic)
5. Chronic renal disease
6. Endocrine deficiencies
7. Selective erythroid aplasia
8. Aplastic and hypoplastic bone marrows

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|---------|---|
| 5.5.1.2 | <ul style="list-style-type: none"><li>9. Lead toxicity (may not be anemic)</li><li>10. Cobalamin deficiency</li></ul> <p><b>Macrocytic Hypochromic</b></p> <ul style="list-style-type: none"><li>1. Regenerative anemias with marked reticulocytosis</li><li>2. Hereditary stomatocytosis in dogs (often slight reticulocytosis)</li><li>3. Abyssinian and Somali cats with increased erythrocyte osmotic fragility (a reticulocytosis is usually present)</li><li>4. Spurious with prolonged storage of blood sample</li><li>5. Spurious in cats and dogs with persistent hypernatremia</li></ul>  |
| 5.5.1.3 | <p><b>Macrocytic Normochromic</b></p> <ul style="list-style-type: none"><li>1. Regenerative anemias (decreased MCHC is not always present)</li><li>2. FeLV infections with no reticulocytosis (common)</li><li>3. Erythroleukemia (AML-M6) and myelodysplastic syndromes</li><li>4. Nonregenerative immune-mediated anemia and/or myelofibrosis in dogs</li><li>5. Poodle macrocytosis (healthy miniature poodles with no anemia)</li><li>6. Cats with hyperthyroidism (slight macrocytosis without anemia)</li><li>7. Folate deficiency (rare)</li><li>8. Congenital dyserythropoiesis of Hereford calves</li><li>9. Spurious with erythrocyte agglutination (MCHC generally increased also)</li></ul> |
| 5.5.1.4 | <p><b>Microcytic Normochromic/Hypochromic<sup>*</sup></b></p> <ul style="list-style-type: none"><li>1. Chronic iron deficiency (months in adults, weeks in nursing animals)</li><li>2. Portosystemic shunts in dogs and cats (often not anemic)</li><li>3. Anemia of inflammatory disease (usually normocytic)</li><li>4. Hepatic lipidosis in cats (usually normocytic)</li><li>5. Normal Akita and Shiba dogs (not anemic)</li><li>6. Prolonged recombinant erythropoietin treatment (mild)</li></ul>   |

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7. Copper deficiency (rare)
8. Drugs or compounds that inhibit heme synthesis
9. Myeloproliferative disorders with abnormal iron metabolism (rare)
10. Pyridoxine deficiency (experimental)
11. Familial dyserythropoiesis of English springer spaniel dogs (rare)
12. Hereditary elliptocytosis in dogs (rare)
13. Spurious when platelets are included in erythrocyte histograms
14. Spurious in dogs with persistent hyponatremia

\* The presence of low MCHC along with low mean cell volume strongly suggests iron deficiency anemia.

*AML-M6*, Acute myeloid leukemia-M6; *FeLV*, feline leukemia virus; *MCHC*, mean cell hemoglobin concentration.

### 5.5.2 BOX 4-3 Causes of Hemolytic Anemias in Domestic Animals

1. Immune-mediated erythrocyte destruction—Autoimmune hemolytic anemia (primarily dogs); neonatal isoerythrolysis (primarily horses and cats); systemic lupus erythematosus (primarily dogs); incompatible blood transfusions; drugs including penicillin (horses), cephalosporins (dogs), levamisole (dogs), sulfonamides (horses), pirimicarb (insecticide in dogs), propylthiouracil (cats), and possibly griseofulvin and albendazole (cats)
2. Erythrocyte parasites (may have an immune-mediated component)—*Anaplasma* spp. (ruminants), hemotropic *Mycoplasma* spp. (cats, dogs, pigs, and sheep), *Babesia* spp., *Cytauxzoon felis*, *Theileria* spp. (ruminants)
3. Other infectious agents (may have an immune-mediated component)—*Leptospira* and *Clostridium* spp. (primarily ruminants and horses), FeLV (at times), equine infectious anemia virus (acute stage of infection), *Sarcocystis* spp. (cattle and sheep), *Trypanosoma* spp. (primarily outside the United States)
4. Chemicals and plants (most are oxidants)—Onions, red maple (horses), *Brassica* spp. (ruminants), lush winter rye (cattle), copper (sheep and goats), phenothiazine (horses), acetaminophen (cats and dogs), methylene blue (cats and dogs), benzocaine (cats and dogs), phenazopyridine (cats), methionine (cats), vitamin K (dogs), propylene glycol (cats), naphthalene (dogs?), zinc (dogs and ruminants), indole (experimental in cattle and horses), tryptophan (experimental in horses), crude oil (marine birds), snake venoms
5. Fragmentation—Disseminated intravascular coagulation (primarily dogs), dirofilariasis (especially vena caval syndrome) in dogs, hemangiosarcoma (dogs), vasculitis, hemolytic uremia syndrome
6. Hypo-osmolality—Hypotonic fluid administration, water intoxication (primarily in cattle)

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7. Hypophosphatemia—Postparturient hemoglobinuria (cattle), ketoacidotic diabetic animals after insulin therapy (cats and dogs), hepatic lipidosis (cats), hyperalimentation (small animals)
8. Hereditary erythrocyte defects—Pyruvate kinase deficiency (dogs and cats), phosphofructokinase deficiency (dogs), glucose-6-phosphate dehydrogenase deficiency (horses), hereditary stomatocytosis (mild anemia in dogs), erythropoietic porphyria (cattle and cats), hereditary nonspherocytic hemolytic anemias of unknown etiology (poodles and beagles), idiopathic increased erythrocyte osmotic fragility (cats), erythrocyte flavin adenine dinucleotide deficiency in horses (methemoglobinemia and sometimes mild anemia), hereditary spherocytosis in cattle and dogs
9. Miscellaneous—Liver failure (horses), hypersplenism, splenic torsion (dogs), selenium deficiency in cattle grazing on St. Augustine grass, postparturient hemoglobinuria in cattle not associated with hypophosphatemia

An increase in the plasma bilirubin concentration imparts a yellow color to the plasma. Hyperbilirubinemia associated with a substantial decrease in the HCT suggests increased phagocytosis of erythrocytes.

If substantial intravascular hemolysis occurs rapidly, hemoglobinemia and, subsequently, hemoglobinuria may be observed. Disorders in which significant intravascular hemolysis sometimes occurs include immune-mediated hemolytic anemia, oxidant chemical and plant toxicities, severe hypophosphatemia, leptospiral and clostridial infections, coral snake and rattlesnake envenomation, zinc toxicity, copper toxicity, babesiosis, hypo-osmolality, vena caval syndrome of dirofilariasis in dogs, hepatic failure in horses, phosphofructokinase deficiency in dogs, inherited idiopathic increased erythrocyte osmotic fragility in Abyssinian and Somali cats, postparturient cattle without hypophosphatemia, and splenic torsion in dogs. In most of these disorders, however, erythrocyte destruction occurs primarily by increased phagocytosis.

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## 5.6 BLOOD LOSS ANEMIAS

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Causes of blood loss anemia are listed in [Box 4-4](#). In some cases, the diagnosis of blood loss anemia and its cause are apparent from the history and/or physical findings. In other cases, hemorrhage is apparent, but its cause must be determined. Finally, blood loss anemia and its cause may not be recognized until laboratory and other diagnostic tests are done. The gastrointestinal and urogenital tracts are common sites of occult hemorrhage. Tests that may assist in the diagnosis of gastrointestinal hemorrhage include the occult blood test in feces, fecal examination for parasite ova, and diagnostic imaging to identify tumors or ulcers. Urinalysis and diagnostic imaging of the urinary tract may assist in the diagnosis of renal or bladder hemorrhage.

### 5.6.1 External Hemorrhage

Although total blood volume is decreased, HCT and plasma protein concentration are normal immediately after substantial acute blood loss has occurred because there is a balanced loss of erythrocytes and plasma. The HCT may even be increased shortly after acute blood loss in horses and dogs because splenic contraction occurs, which releases blood with a higher HCT into the general circulation. After several hours, the HCT and plasma protein concentration decrease as the animal drinks and as fluid moves from extravascular spaces into the circulation to return the blood volume toward normal. If no further hemorrhage occurs, the plasma protein concentration will return to normal within 5 to 7 days. Consequently, the occurrence of a low plasma protein concentration in association with anemia suggests the presence of recent or ongoing hemorrhage. Considerably more time is required for the HCT to return to normal than is required for the plasma protein concentration to

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return to normal. After blood loss that reduces the HCT by half, the HCT increases approximately 0.4%, 0.8%, and 1.6% units per day in horses, cats, and dogs, respectively.

5.6.1.1

## BOX 4-4 Causes of Blood Loss Anemias in Domestic Animals

1. Trauma—Accidents, fights, gastrointestinal foreign bodies, surgery
2. Parasites—Hookworms, fleas, blood-sucking lice, *Haemonchus* spp., *Coccidia* spp.
3. Coagulation disorders—Vitamin K deficiency, sweet clover dicumarol toxicity (cattle), rodenticide toxicity, bracken fern toxicity (cattle), disseminated intravascular coagulation, inherited coagulation factor deficiencies (see [Chapter 6](#))
4. Platelet disorders—Thrombocytopenia and inherited platelet function defects (see [Chapter 6](#))
5. Neoplasia—Gastric tumors including carcinomas and leiomyosarcomas, transitional cell carcinoma of the bladder (dogs), and ruptured hemangiosarcoma and adrenal gland tumors with bleeding into body cavities and tissues (dogs)
6. Gastrointestinal ulcers—Glucocorticoids, nonsteroidal antiinflammatory drugs, mast cell tumor, gastrinoma, stress, metabolic diseases (uremia, liver failure, hypoadrenocorticism)
7. Inflammatory bowel diseases

The anemia appears nonregenerative shortly after blood loss because approximately 4 days are required for production of reticulocytes by the marrow. The MCV may not be increased after blood loss in animals because the reticulocyte response may not be of sufficient magnitude to result in a high MCV. Few reticulocytes are released from the marrow in response to blood loss anemia in cattle, and no reticulocytes are released after hemorrhage in horses.

Chronic external blood loss can result in iron deficiency. Iron deficiency anemia is common in adult dogs and ruminants but seldom occurs in adult cats and horses because parasitism causing significant blood loss is uncommon in these species. If iron deficiency persists for several weeks, the anemia can become microcytic and hypochromic. Reticulocyte counts may be slightly to moderately increased in early iron deficiency anemia in dogs; however, as iron deficiency becomes more severe, the regenerative response will be attenuated (see discussion of iron deficiency later in this chapter).

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5.6.2

## Internal Hemorrhage

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Hemorrhage may occur into body cavities or tissues. This internal hemorrhage may share some characteristics of hemolytic anemias. Iron is conserved so that hypoferrremia does not occur. Some plasma proteins and erythrocytes may be reabsorbed when hemorrhage occurs in body cavities. Consequently, the total plasma protein concentration may be only transiently decreased. Slight hyperbilirubinemia may occur as a result of phagocytosis and degradation of erythrocytes at the site of hemorrhage.

5.7

## DECREASED ERYTHROCYTE PRODUCTION ANEMIAS

Anemias resulting from decreased erythrocyte production lack evidence of bone marrow response to the anemia (e.g., the absolute reticulocyte count in blood is not increased or is only minimally increased for the degree of

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anemia). Nonregenerative anemias result from reduced or defective erythropoiesis (Box 4-5). They are usually normocytic. Exceptions include microcytic anemia associated with chronic iron deficiency anemia, copper deficiency, pyridoxine deficiency, and dyserythropoiesis in English springer spaniels and macrocytic anemia associated with folate deficiency, FeLV infection in cats, erythroleukemia, some myelodysplastic disorders, and dyserythropoiesis in polled Hereford calves. Bone marrow biopsies are often required to delineate the nature of nonregenerative anemias.

### 5.7.1 BOX 4-5 Anemias Resulting from Decreased Erythrocyte Production in Domestic Animals

#### 5.7.1.1 Reduced Erythropoiesis

1. Chronic renal disease—Primarily lack of erythropoietin
2. Endocrine deficiencies—Hypothyroidism, hypoadrenocorticism, hypopituitarism, hypoandrogenism
3. Inflammatory disease—Inflammation and neoplasia
4. Cytotoxic damage to the marrow—Bracken fern poisoning (cattle), cytotoxic anticancer drugs, estrogen toxicity (dogs and ferrets), chloramphenicol (cats, usually not anemic), phenylbutazone (dogs), trimethoprim-sulfadiazine (dogs), radiation, albendazole (dogs), griseofulvin (cats), trichloroethylene (cattle)
5. Infectious agents—*Ehrlichia* spp. (dogs, horses, and cats), FeLV, nonbloodsucking trichostrongyloid parasites (ruminants), parvovirus (canine pups)
6. Immune-mediated—Nonregenerative anemia, selective erythroid aplasia, continued treatment with recombinant human erythropoietin, idiopathic aplastic anemia (?)
7. Congenital/Inherited—Calves and foals?
8. Myelophthisis—Myelogenous leukemias, lymphoid leukemias, myelodysplastic syndromes, multiple myeloma, myelofibrosis, osteosclerosis, metastatic lymphomas, metastatic mast cell tumors

#### 5.7.1.2 Defective Erythropoiesis

1. Disorders of heme synthesis—Iron, copper, and pyridoxine deficiencies; lead toxicity; drugs
2. Disorders of nucleic acid synthesis—Folate and cobalamin deficiencies
3. Abnormal maturation—Erythroleukemia or AML-M6 (primarily cats), myelodysplastic syndromes with erythroid predominance (MDS-Er), inherited dyserythropoiesis of Hereford calves, inherited dyserythropoiesis of English springer spaniels, chemotherapeutic drugs such as vincristine, idiopathic in dogs

*AML-M6*, Acute myeloid leukemia-M6; *FeLV*, feline leukemia virus.

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## 5.7.2 Nonregenerative Anemias without Leukopenia or Thrombocytopenia

A nonregenerative anemia without an accompanying leukopenia or thrombocytopenia in blood suggests a bone marrow abnormality affecting only erythroid cells. Mild to moderate anemia of this type may occur in association with chronic renal disease, endocrine deficiencies, and the anemia of inflammatory disease. Erythroid production is reduced in these disorders, but often not enough to result in an M/E ratio in the marrow that is increased above the reference interval.

### 5.7.2.1 Hormone Deficiencies

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Because the kidneys are the major site of EPO production in the body, chronic renal disease can result in a mild to moderate nonregenerative anemia associated with reduced EPO production. Disorders such as hypopituitarism, hypoadrenocorticism, hypothyroidism, and hypoandrogenism may result in mild nonregenerative anemia because these hormones apparently increase the number of erythroid colonies formed, possibly by modulating the fraction of erythroid progenitor cells that will enter terminal differentiation at a given EPO concentration.

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### 5.7.2.2 Anemia of Inflammatory Disease (Anemia of Chronic Disease)

A mild to moderate nonregenerative anemia often accompanies chronic inflammatory and neoplastic disorders. The cause of the anemia is multifactorial and only partially understood. Abnormalities that can contribute to the anemia include low serum iron values, the production of inflammatory mediators that can inhibit erythropoiesis, and shortened erythrocyte life spans, presumably associated with membrane damage caused by endogenous oxidants generated during inflammation.

### 5.7.2.3 Disorders of Nucleic Acid Synthesis

Anemias resulting from folate deficiency are rarely reported in animals. Macrocytic anemia has been produced experimentally in pigs, and a clinical case of folate deficiency has been recognized in a cat. Cobalamin (vitamin B<sub>12</sub>) deficiency in humans causes hematologic abnormalities similar to those associated with folate deficiency because cobalamin is necessary for normal folate metabolism in humans. In contrast, cobalamin deficiency does not cause macrocytic anemia in any animal species. Anemia has been reported in some experimental animal studies, but erythrocytes were of normal size. Cobalamin deficiency occurs as a result of an inherited malabsorption of cobalamin in dogs. Affected animals have normocytic, nonregenerative anemia with increased anisocytosis. Additional findings include neutropenia with hypersegmented neutrophils and giant platelets. A normocytic nonregenerative anemia was also present in a cobalamin-deficient cat that probably had an inherited defect in cobalamin absorption.

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Table 4-1 Laboratory Findings in Chronic Iron Deficiency Anemia Versus the Anemia of Inflammatory Disease

Measurement	Chronic Iron Deficiency	Anemia of Inflammatory Disease
HCT	Slight to marked decrease	Slight to moderate decrease
MCV	Slight to marked decrease	Normal to slight decrease
Serum iron	Slight to marked decrease	Slight to moderate decrease
Serum TIBC	Normal to increased	Normal to decreased
Serum ferritin	Decreased	Normal to increased
Marrow hemosiderin	Decreased or absent	Normal to increased

*HCT*, Hematocrit; *MCV*, mean cell volume; *TIBC*, total iron-binding capacity.

## 5.7.2.4 Abnormalities in Heme Synthesis

Iron deficiency in adult domestic animals usually results from blood loss. The absolute reticulocyte count may be increased early in response to hemorrhage, but as iron deficiency becomes more severe, minimal regenerative response occurs. Microcytic erythrocytes form when iron becomes limiting because erythroid cells undergo additional divisions resulting in smaller than normal cells. If sufficient time has elapsed for these small cells to account for a substantial portion of the total erythrocyte population, the MCV will decrease below the normal reference interval. When the MCV is only slightly decreased, the MCHC is usually normal. When the MCV is substantially below normal, the MCHC will also be decreased (hypochromic). Erythrocytes in these microcytic hypochromic anemias will appear hypochromic (pale cells with prominent areas of central pallor) on stained blood films. Hematologic aspects of iron deficiency are compared with the anemia of inflammatory disease in [Table 4-1](#).

Milk contains little iron; consequently, nursing animals can deplete body iron stores as they grow. Microcytic erythrocytes are produced in response to iron deficiency, but a low MCV may not develop postnatally in species such as dogs and cats in which the MCV is greater than adult values at birth. The potential for development of severe iron deficiency in young animals appears to be less likely in species that begin to eat solid food at an early age. Piglets are especially susceptible to the development of iron deficiency when they are not raised in pens with dirt floors; thus, iron injections are routinely given to piglets raised in pens with slatted floors.

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Prolonged copper deficiency usually results in anemia in mammals. Because copper is required for normal iron metabolism, the anemia that develops is generally microcytic, but it may be normocytic. Pyridoxine (vitamin B6) is required for the first step in heme synthesis. Although natural cases of pyridoxine deficiency have not been documented in domestic animals, microcytic anemias with high serum iron values have been produced experimentally in dogs, cats, and pigs with dietary pyridoxine deficiency.

## 5.7.2.5 Nonregenerative Immune-Mediated Anemia

Erythroid cellularity in the marrow varies from hypocellular to hypercellular in dogs with nonregenerative immune-mediated anemia. Erythroid maturation may be complete to the polychromatophilic erythrocyte stage, or a maturation arrest may occur at an earlier stage of erythrocyte development. An antibody or cell-mediated response may be directed against one or more maturation antigens present on nucleated erythrocyte precursors and/or reticulocytes.

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## 5.7.2.6 Selective Erythroid Aplasia

Pure red cell aplasia or selective erythroid aplasia can result in severe anemia in dogs and cats. Most cases appear to be acquired, but congenital erythroid aplasia may occur in dogs. Some cases in adult dogs and cats appear to be immune-mediated. Erythroid hypoplasia or dysplasia is also reported to be a rare sequela of vaccination against parvovirus in dogs. Selective erythroid aplasia occurs in cats infected with FeLV subgroup C, but not in cats infected only with subgroups A or B. High doses of chloramphenicol cause reversible erythroid hypoplasia in some dogs and erythroid aplasia in cats. The continued use of a recombinant human erythropoietin (EPO) can result in erythroid aplasia in animals, when antibodies made against the recombinant EPO also neutralize the endogenous EPO of the species receiving treatment.

## 5.7.2.7 Dyserythropoiesis

Abnormal erythrocyte maturation occurs as a component of inherited disorders in Hereford calves and English springer spaniels. Dyserythropoiesis may occur after treatment with certain chemotherapeutic drugs, such as vincristine, and as an idiopathic disorder in dogs. Megaloblastic erythroid cells are often seen in the bone marrow of animals (especially cats) with erythroleukemia and myelodysplastic syndromes with erythroid predominance; however, leukopenia, thrombocytopenia, or both are usually present along with nonregenerative anemia in these disorders.

## 5.7.3 Nonregenerative Anemias with Leukopenia and/or Thrombocytopenia

A pattern of nonregenerative anemia with leukopenia and/or thrombocytopenia suggests that either the marrow is hypocellular or high numbers of abnormal cells have replaced the normal hematopoietic precursors (myelophthisis). Pancytopenia may occasionally be present in disorders other than marrow hypoplasia or aplasia and myelophthisis. Examples include the terminal stage of cytauxzoonosis in cats and septicemia in anemic animals. These disorders involve the peripheral utilization or destruction of blood cells.

## 5.7.3.1 Hypocellular/Aplastic Bone Marrow

When more than 75% of the marrow sample from an adult animal is composed of fat, it is considered to be hypocellular. When all hematopoietic cell types—erythrocytic, granulocytic, and megakaryocytic—are markedly reduced or absent, the marrow is said to be aplastic. Anemic animals with generalized marrow aplasia are reported to have aplastic anemia. When only one cell line is reduced or absent, a more restrictive term, such as *granulocytic hypoplasia* or *erythroid aplasia*, is used to describe the abnormalities present. Hypocellular or aplastic bone marrow may result from insufficient numbers of stem cells, abnormalities in the hematopoietic microenvironment, or abnormal humoral or cellular control of hematopoiesis. These factors are interrelated, and the specific defect in a given disorder is usually unknown.

Drug-induced causes of aplastic anemia or generalized marrow hypoplasia in animals include estrogen toxicity in dogs, phenylbutazone toxicity in dogs (and possibly horses), administration of trimethoprim-sulfadiazine to dogs, bracken fern poisoning in cattle and sheep, ingestion of trichloroethylene-extracted soybean meal by cattle, albendazole toxicity in dogs, griseofulvin toxicity in cats, treatment with various cancer chemotherapeutic agents, and radiation. Thiacetarsamide, meclofenamic acid, and quinidine have also been suggested as potential causes of aplastic anemia in dogs.

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In addition to exogenous estrogen injections, high levels of endogenous estrogens produced by Sertoli cell, interstitial cell, and granulosa cell tumors can cause aplastic anemia in dogs. Functional cystic ovaries also have the potential to induce myelotoxicity in dogs. Ferrets have induced ovulations and may remain in estrus for long periods when they are not bred. This prolonged exposure to a high endogenous estrogen concentration can result in aplastic anemia.

Parvovirus infections can cause erythroid hypoplasia, as well as myeloid hypoplasia in canine pups, but animals may not become anemic because of the long life span of erythrocytes. Thrombocytopenia is mild or absent because megakaryocytes are still present in the bone marrow. In contrast to its effects in pups, parvovirus is reported to have a minimal effect on erythroid progenitors in adult dogs. Only leukopenia associated with myeloid hypoplasia is typically present in cats with parvovirus (panleukopenia virus) infection.

Although some degree of marrow hypoplasia and/or dysplasia often occurs in cats with FeLV infections, true aplastic anemia is not a well-documented sequela. Hypocellular bone marrow has been reported in experimental cats infected with both FeLV and feline parvovirus.

Dogs with acute *Ehrlichia canis* infections may spontaneously recover or have chronic disease that generally exhibits some degree of marrow hypoplasia. Although rare, aplastic anemia may develop in association with severe chronic ehrlichiosis in dogs.

Congenital aplastic anemia has been reported in foals and a calf. Treatment of dams for equine protozoal myeloencephalitis with sulfonamides and/or pyrimethamine during pregnancy may have caused the aplastic anemia in some foals. Generalized bone marrow hypoplasia has been reported in eight young standard-bred horses sired by the same stallion, suggesting an inherited etiology.

Idiopathic aplastic anemia has also been reported in dogs, cats, and horses. A primary immune-mediated reaction directed against hematopoietic precursor cells has been proposed as a cause of aplastic anemia in dogs.

5.7.3.2

## Myelophthistic Disorders

Myelophthistic disorders are characterized by the replacement of normal hematopoietic cells with abnormal ones. Examples include myelogenous leukemias; lymphoid leukemias; multiple myeloma; myelodysplastic syndromes; myelofibrosis (often associated with anemia, but less often with pancytopenia); osteosclerosis; and metastasis of lymphomas, carcinomas, and mast cell tumors. Myelophthistic disorders do not simply “crowd out” normal cells, they also alter the marrow microenvironment so that normal hematopoiesis is compromised. In the case of myelodysplastic syndromes, increased apoptosis (programmed cell death) probably accounts for the ineffective hematopoiesis that is present.

5.7.4

## Physiologic Anemia of Neonatal Animals

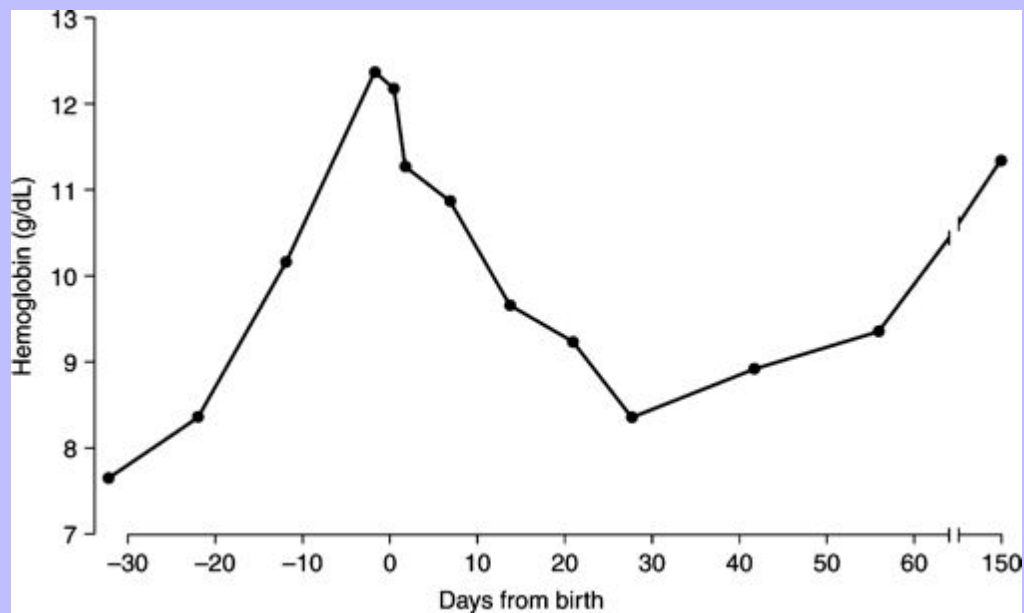
HCT and hemoglobin values increase during fetal development, reaching values near those of adult animals at birth (Fig. 4-14). After birth, there is a rapid decrease in these values during the first few weeks of life, which is followed by a gradual increase to adult values by 4 months of age in most species (Fig. 4-15). Factors involved in the development of the anemia of the neonate include absorption of colostric proteins during the first day of life (increases plasma volume through an osmotic effect); decreased erythrocyte production during the early neonatal period; shortened life span of erythrocytes formed in utero; and rapid growth with hemodilution resulting from total plasma volume expansion, which occurs more rapidly than the increase in total erythrocyte mass.

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In some species, production of erythrocytes is decreased because of low EPO concentrations at birth. The decreased stimulus for EPO production at birth may occur as a result of a placental blood transfusion that increases erythrocyte mass immediately after birth, a rapid increase in  $\text{PaO}_2$  associated with breathing air, and a decrease in hemoglobin  $\text{O}_2$  affinity caused by an increase in erythrocyte 2,3-DPG content after birth. Although not involved in the early, rapid decrease in HCT, iron availability may limit the response to anemia in some rapidly growing animals.

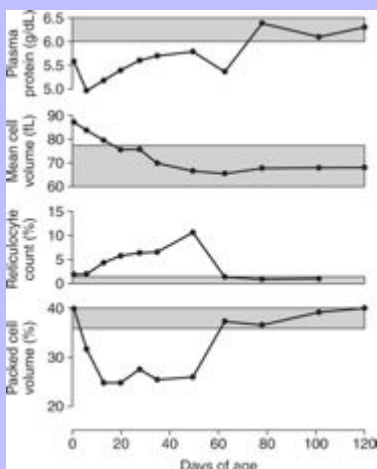
Fig. 4-14



Blood hemoglobin values in prenatal and postnatal cats. The graph includes data from Windle WF, Sweet M, Whitehead WH: Some aspects of prenatal and postnatal development of the blood of cats. Anat Rec 1940;78:321.

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Fig. 4-15



Age-related changes in total plasma protein concentration, mean cell volume, raw reticulocyte count, and packed cell volume in blood from a Basenji dog.

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## 5.8 ERYTHROCYTOSIS (POLYCYTHEMIA)

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*Erythrocytosis* refers to an increase in HCT, hemoglobin, and RBC count above the normal reference interval. The reference interval can sometimes vary by breed, as well as by species. The HCTs of hot-blooded horses (e.g., thoroughbreds, quarter horses, and Arabians) are usually higher than those of draft horses because of much larger spleens in the hot-blooded group. The reference interval for the HCT in greyhounds (49% to 65%) is higher than that of other breeds. In addition, slightly increased HCTs are occasionally measured in individuals from certain breeds of dogs (e.g., poodle, German shepherd, boxer, beagle, dachshund, and Chihuahua). These somewhat higher values are believed to result from splenic contraction in animals with a high normal erythrocyte mass.

### 5.8.1 Relative Erythrocytosis

Erythrocytosis is either relative (spurious) or absolute (Box 4-6). A relative erythrocytosis is one in which the HCT is high but the total erythrocyte mass is normal. It is caused by splenic contraction or dehydration. Splenic contraction is stimulated by epinephrine release, as occurs with excitement, fear, pain, or exercise. The HCT measured in blood from peripheral veins increases because the HCT in the spleen is considerably higher than that in the general circulation. Increases in HCT are pronounced in hot-blooded horses, which have especially large, contractile spleens.

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#### 5.8.1.1 BOX 4-6 Erythrocytosis in Domestic Animals

##### 5.8.1.1.1 Relative Erythrocytosis

1. Splenic contraction—Excitement, exercise, pain (primarily in horses, dogs, and cats)

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5.8.1.1.2

## Absolute Erythrocytosis

2. Dehydration—Water loss, water deprivation, shock with fluid shift into tissues
1. Primary erythrocytosis—A myeloproliferative disorder in adult dogs and cats
2. Familial erythrocytosis in young Jersey cattle—Etiology unknown
3. Hypoxemia with compensatory increased erythropoietin production—High altitude, chronic lung disease, heart disease with right-to-left shunting of blood, chronic methemoglobinemia (rare in dogs and cats)
4. Inappropriate erythropoietin production—Renal tumors, renal cysts, hydronephrosis (rare), nonrenal erythropoietin secreting tumors (rare)

Dehydration results from increased water loss (diarrhea, vomiting, excessive diuresis, or sweating) or from water deprivation. The plasma protein concentration is also usually increased. The HCT may also be high when increased vascular permeability results in protein and water loss from the circulation into the tissues, as occurs in endotoxic shock.

5.8.2

## Absolute Erythrocytosis

An absolute erythrocytosis is one in which the HCT is high because the total erythrocyte mass in the body is increased. Absolute erythrocytosis may occur as a result of increased EPO production (secondary erythrocytosis) or in disorders in which increased erythrocyte proliferation occurs in the presence of normal or low blood EPO values (primary erythrocytosis). Causes of secondary erythrocytosis include chronic hypoxemia (heart defects with right-to-left shunting of blood, chronic lung disease, high altitude, methemoglobinemia); renal disorders causing local tissue hypoxia (renal tumors, renal cysts, hydronephrosis); and tumors that secrete EPO, EPO-like proteins, or other hormones such as androgens that might enhance the effects of EPO.

Primary erythrocytosis (polycythemia vera) is considered to be a chronic myeloproliferative disorder that results from an autonomous (EPO-independent) proliferation of erythroid precursor cells, resulting in high numbers or mature erythrocytes in blood. In contrast to humans with polycythemia vera, animals do not generally have increased blood granulocyte and platelet numbers; consequently, the term *primary erythrocytosis* is more appropriate than the term *polycythemia vera* in veterinary medicine. Bone marrow is hyperplastic, and maturation of erythroid cells is orderly in primary erythrocytosis. The M/E ratio is often normal but may be decreased as a result of erythroid hyperplasia. A diagnosis of primary erythrocytosis is ultimately made by ruling out causes of secondary erythrocytosis.

Familial erythrocytosis (HCTs of 60% to 80%) has been described in calves from a highly inbred Jersey herd. The cause of this defect was not determined. Affected calves had normal hemoglobin types and arterial blood gas values and lacked measurable EPO in plasma. The majority of affected calves died by 6 months of age. HCTs of surviving animals slowly returned to normal by maturity.

5.8.3

## Differential Diagnosis of Erythrocytosis

Splenic contraction is considered a likely cause of erythrocytosis when the HCT is slightly to moderately increased in the absence of evidence of dehydration. A slight to moderate increase in HCT with increased plasma

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protein concentration suggests that dehydration is present. This interpretation is confirmed by finding evidence of dehydration on physical examination.

The persistence of a moderate or marked increase in HCT suggests that an absolute erythrocytosis is present. Tests that may help determine the cause of the absolute erythrocytosis include arterial blood gas measurements, diagnostic imaging, a methemoglobin screening test, and a validated EPO test. The cytologic examination of bone marrow is not useful. When present, methemoglobinemia is easily detected with the use of a simple spot test (see previous methemoglobinemia section). The presence of low Pao<sub>2</sub> suggests that either a heart defect

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(with right-to-left shunting of blood) or chronic lung disease is present. Diagnostic imaging procedures are used to differentiate heart and lung disease and search for renal lesions. Plasma EPO values should be increased when hypoxemia, renal lesions, or EPO-secreting tumors cause the erythrocytosis, but are low when primary erythrocytosis is present. A diagnosis of primary erythrocytosis is reached after other potential causes of persistent erythrocytosis have been ruled out.

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## 5.9 ADDITIONAL READING

B Aird: Acute blood loss. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 151–153.

GA Andrews, JE Smith: Iron metabolism. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 129–139.

NC Andrews: Disorders of iron metabolism. *N Engl J Med*. **341**, 1999, 1986–1995.

NL Bailiff, CR Norris: Clinical signs, clinicopathological findings, etiology, and outcome associated with hemoptysis in dogs: 36 cases (1990–1999). *J Am Anim Hosp Assoc*. **38**, 2002, 125–133.

M Beppu, K Ando, K Kikugawa: Poly-N-acetyllactosaminyl saccharide chains of band 3 as determinants for anti-band 3 autoantibody binding to senescent and oxidized erythrocytes. *Cell Mol Biol*. **42**, 1996, 1007–1024.

AM Boisvert, HW Tvedten, MA Scott: Artifactual effects of hypernatremia and hyponatremia on red cell analytes measured by the Bayer H\*1 Analyzer. *Vet Clin Pathol*. **28**, 1999, 91–96.

JD Boyer, DC Breeden, DL Brown: Isolation, identification, and characterization of compounds from *Acer rubrum* capable of oxidizing equine erythrocytes. *Am J Vet Res*. **63**, 2002, 604–610.

D Bratosin, J Mazurier, JP Tissier, et al.: Cellular and molecular mechanisms of senescent erythrocyte phagocytosis by macrophages. A review. *Biochimie*. **80**, 1998, 173–195.

EB Breitschwerdt, AC Abrams-Ogg, MR Lappin, et al.: Molecular evidence supporting *Ehrlichia canis*-like infection in cats. *J Vet Intern Med*. **16**, 2002, 642–649.

W Breuer, Darbès, J, W Hermanns, et al.: Idiopathic myelofibrosis in a cat and in three dogs. *Comp Haematol Int*. **9**, 1999, 17–24.

H Brommer, MM Oldruitenborgh-Oosterbaan: Iron deficiency in stabled Dutch warmblood foals. *J Vet Intern Med*. **15**, 2001, 482–485.

MD Cappellini, D Tavazzi, L Duca, et al.: Metabolic indicators of oxidative stress correlate with haemichrome attachment to membrane, band 3 aggregation and erythrophagocytosis in  $\beta$ -thalassaemia intermedia. *Br J Haematol*. **104**, 1999, 504–512.

I Cavill: The rejected reticulocyte. *Br J Haematol*. **84**, 1993, 563–565.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- M Cazzola, R Guarnone, P Cerani, et al.: Red blood cell precursor mass as an independent determinant of serum erythropoietin level. *Blood*. **91**, 1998, 2139–2145.
- ME Conrad, JN Umbreit: Iron absorption and transport—An update. *Am J Hematol*. **64**, 2000, 287–298.
- LD Cowgill, KM James, JK Levy, et al.: Use of recombinant human erythropoietin for management of anemia in dogs and cats with renal failure. *J Am Vet Med Assoc*. **212**, 1998, 521–528.
- L El Bahri, J Belguith, A Blouin: Toxicology of nitrates and nitrites in livestock. *Comp Contin Educ Pract Vet*. **19**, 1997, 643–648.
- FR Fernandez, CB Grindem: Reticulocyte response. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 110–116.
- AD Fly, GL Czarnecki-Maulden: Iron bioavailability from hemoglobin and hemin in chick, rat, cat, and dog: A comparative study. *Nutr Res*. **20**, 2000, 237–248.
- JE Foley, NC Pedersen: 'Candidatus *Mycoplasma haemominutum*', a low-virulence eperythrocyclic parasite of cats. *Int J Syst Evol Microbiol*. **51**, 2001, 815–817.
- HH Fordyce, MB Callan, U Giger: Persistent cobalamin deficiency causing failure to thrive in a juvenile beagle. *J Small Anim Pract*. **41**, 2000, 407–410.
- T Ganz: Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood*. **102**, 2003, 783–788.
- SD Gaunt: Hemolytic anemias caused by blood rickettsial agents and protozoa. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 154–162.
- JW George, BA Rideout, SM Griffey, et al.: Effect of preexisting FeLV infection or FeLV and feline immunodeficiency virus coinfection on pathogenicity of the small variant of *Haemobartonella felis* in cats. *Am J Vet Res*. **63**, 2002, 1172–1178.
- U Giger: Erythrocyte phosphofructokinase and pyruvate kinase deficiencies. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 1020–1025.
- JL Gookin, SE Bunch, LJ Rush, et al.: Evaluation of microcytosis in 18 Shibas. *J Am Vet Med Assoc*. **212**, 1998, 1258–1259.
- KA Gossett: Anemias associated with drugs and chemicals. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 185–189.
- RA Green: Spurious platelet effects on erythrocyte indices using the Cell-Dyne 3500 Automated Hematology System. *Vet Clin Pathol*. **28**, 1999, 47–49.
- AS Hammer, M Wellman: Leukoerythroblastosis and normoblastemia in the cat. *J Am Anim Hosp Assoc*. **35**, 1999, 471–473.
- JW Harvey: Atlas of veterinary hematology. In *Blood and bone marrow of domestic animals*. 2001, W.B. Saunders, Philadelphia.
- JW Harvey: The erythrocyte: Physiology, metabolism and biochemical disorders. In Kaneko, JJ, Harvey, JW, Bruss, ML (Eds.): *Clinical biochemistry of domestic animals*. 5th ed., 1997, Academic Press, San Diego, 157–203.
- JW Harvey: Hereditary methemoglobinemia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 1008–1011.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

JW Harvey: Methemoglobinemia and Heinz-body hemolytic anemia. In Bonagura, JD (Ed.): <i>Kirk's current veterinary therapy XII. Small animal practice</i> . 1995, WB Saunders, Philadelphia, 443–446.	
JW Harvey: Microcytic anemias. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): <i>Schalm's veterinary hematology</i> . 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 200–204.	
JW Harvey, SL Stockham, Johnson, PJ, et al.: Methemoglobinemia and eccentrocytosis in a horse with erythrocyte flavin adenine dinucleotide (FAD) deficiency (abstract). <i>Rev Med Vet (Toulouse)</i> . <b>151</b> , 2000, 710.	79 80
AH Hasler, U Giger: Serum erythropoietin values in polycythemic cats. <i>J Am Anim Hosp Assoc</i> . <b>32</b> , 1996, 294–301.	
AS Hill, S O'Neill, QR Rogers, et al.: Antioxidant prevention of Heinz body formation and oxidant injury in cats. <i>Am J Vet Res</i> . <b>62</b> , 2001, 370–374.	
M Hisasue, H Okayama, T Okayama, et al.: Hematologic abnormalities and outcome of 16 cats with myelodysplastic syndromes. <i>J Vet Intern Med</i> . <b>15</b> , 2001, 471–477.	
S Hyypya, M Hoyhtya, M Nevalainen, et al.: Effect of exercise on plasma ferritin concentrations: Implications for the measurement of iron status. <i>Equine Vet J Suppl</i> . <b>34</b> , 2002, 186–190.	
M Inaba, A Yawata, I Koshino, et al.: Defective anion transport and marked spherocytosis with membrane instability caused by hereditary total deficiency of red cell band 3 in cattle due to a nonsense mutation. <i>J Clin Invest</i> . <b>97</b> , 1996, 1804–1817.	
GJ Kociba: Macrocytosis. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): <i>Schalm's veterinary hematology</i> . 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 196–199.	
B Kohn, MH Goldschmidt, AE Hohenhaus, et al.: Anemia, splenomegaly, and increased osmotic fragility of erythrocytes in Abyssinian and Somali cats. <i>J Am Vet Med Assoc</i> . <b>217</b> , 2000, 1483–1491.	
LI Kushner, AJ Mackin: Anesthesia case of the month. Cyanosis in a dog during anesthesia. <i>J Am Vet Med Assoc</i> . <b>214</b> , 1999, 1007–1009.	
KW Lee, O Yamato, M Tajima, et al.: Hematologic changes associated with the appearance of eccentrocytes after intragastric administration of garlic extract to dogs. <i>Am J Vet Res</i> . <b>61</b> , 2000, 1446–1450.	
TJ Lennox, JH Wilson, DW Hayden, et al.: Hepatoblastoma with erythrocytosis in a young female horse. <i>J Am Vet Med Assoc</i> . <b>216</b> , 2000, 718–721.	
SM MacNaughton: Acetaminophen toxicosis in a Dalmatian. <i>Can Vet J</i> . <b>44</b> , 2003, 142–144.	
AP Masini, P Baragli, D Tedeschi, et al.: Behavior of mean erythrocyte volume during submaximal treadmill exercise in the horse. <i>Comp Haematol Int</i> . <b>10</b> , 2000, 38–42.	
D McFarlane, DC Sellon, P Parker: Primary erythrocytosis in a 2-year-old Arabian gelding. <i>J Vet Intern Med</i> . <b>12</b> , 1998, 384–388.	
JH Meinkoth, AA Kocan, SD Loud, et al.: Clinical and hematologic effects of experimental infection of dogs with recently identified <i>Babesia gibsoni</i> -like isolates from Oklahoma. <i>J Am Vet Med Assoc</i> . <b>220</b> , 2002, 185–189.	
JB Messick, PG Walker, W Raphael, et al.: ' <i>Candidatus mycoplasma haemodidelphidis</i> ' sp. nov., ' <i>Candidatus mycoplasma haemolamae</i> ' sp. nov. and <i>Mycoplasma haemocanis</i> comb. nov., haemotrophic parasites from a naturally infected opossum ( <i>Didelphis virginiana</i> ), alpaca ( <i>Lama pacos</i> ) and dog ( <i>Canis familiaris</i> ): Phylogenetic and secondary structural relatedness of their 16S rRNA genes to other mycoplasmas. <i>Int J Syst Evol Microbiol</i> . <b>52</b> , 2002, 693–698.	
JR Middleton, L Katz, JA Angelos, et al.: Hemolysis associated with water administration using a nipple bottle for human infants in juvenile pygmy goats. <i>J Vet Intern Med</i> . <b>11</b> , 1997, 382–384.	

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

- DR Monke, GJ Kociba, M DeJarnette, et al.: Reference values for selected hematologic and biochemical variables in Holstein bulls of various ages. *Am J Vet Res.* **59**, 1998, 1386–1391.
- KW Moore, RL Stepien: Hydroxyurea for treatment of polycythemia secondary to right-to-left shunting patent ductus arteriosus in 4 dogs. *J Vet Intern Med.* **15**, 2001, 418–421.
- LW Morgan, J McConnell: Cobalamin deficiency associated with erythroblastic anemia and methylmalonic aciduria in a border collie. *J Am Anim Hosp Assoc.* **35**, 1999, 392–395.
- R Neiger, J Hadley, DU Pfeiffer: Differentiation of dogs with regenerative and non-regenerative anaemia on the basis of their red cell distribution width and mean corpuscular volume. *Vet Rec.* **150**, 2002, 431–434.
- H Neimark, KE Johansson, Y Rikihisa, et al.: Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of 'Candidatus Mycoplasma haemofelis', 'Candidatus Mycoplasma haemomuris', 'Candidatus Mycoplasma haemosuis' and 'Candidatus Mycoplasma wenyonii'. *Int J Syst Evol Microbiol.* **51**, 2001, 891–899.
- A Niggemeier, HF Haberstroh, VE Nelson, et al.: An accidental transfusion of a type A kitten with type B blood causes a transient switch from blood type A to B. *J Vet Intern Med.* **14**, 2000, 214–216.
- SJ Noble, PJ Armstrong: Bee sting envenomation resulting in secondary immune-mediated hemolytic anemia in two dogs. *J Am Vet Med Assoc.* **214**, 1999, 1026–1027.
- D Pechereau, P Martel, JP Braun: Plasma erythropoietin concentrations in dogs and cats: Reference values and changes with anaemia and/or chronic renal failure. *Res Vet Sci.* **62**, 1997, 185–188.
- RJ Piercy, CJ Swardson, KW Hinchcliff: Erythroid hypoplasia and anemia following administration of recombinant human erythropoietin to two horses. *J Am Vet Med Assoc.* **212**, 1998, 244–247.
- P Ponka: Cell biology of heme. *Am J Med Sci.* **318**, 1999, 241–256.
- JG Quigley, CC Burns, MM Anderson, et al.: Cloning of the cellular receptor for feline leukemia virus subgroup C (FeLV-C), a retrovirus that induces red cell aplasia. *Blood.* **95**, 2000, 1093–1099.
- HA Rae: Onion toxicosis in a herd of beef cows. *Can Vet J.* **40**, 1999, 55–57.
- SK Ramaiah, JW Harvey, S Giguère, et al.: Intravascular hemolysis associated with liver disease in a horse with marked neutrophil hypersegmentation. *J Vet Intern Med.* **17**, 2003, 360–363.
- JF Randolph, T Stokol, JM Scarlett, et al.: Comparison of biological activity and safety of recombinant canine erythropoietin with that of recombinant human erythropoietin in clinically normal dogs. *Am J Vet Res.* **60**, 1999, 636–642.
- ME Reimer, GC Troy, LD Warnick: Immune-mediated hemolytic anemia: 70 cases (1988–1996). *J Am Anim Hosp Assoc.* **35**, 1999, 384–391.
- MP Rettig, PS Low, JA Gimm, et al.: Evaluation of biochemical changes during in vivo erythrocyte senescence in the dog. *Blood.* **93**, 1999, 376–384.
- JM Ristic, MF Stidworthy: Two cases of severe iron-deficiency anaemia due to inflammatory bowel disease in the dog. *J Small Anim Pract.* **43**, 2002, 80–83.
- JE Robertson, MM Christopher, QR Rogers: Heinz body formation in cats fed baby food containing onion powder. *J Am Vet Med Assoc.* **212**, 1998, 1260–1266.
- CN Roy, CA Enns: Iron homeostasis: New tales from the crypt. *Blood.* **96**, 2000, 4020–4027.
- K Sato, Y Hikasa, T Morita, et al.: Secondary erythrocytosis associated with high plasma erythropoietin concentrations in a dog with cecal leiomyosarcoma. *J Am Vet Med Assoc.* **220**, 2002, 486–490, 464.

80

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# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- T Shimoda, N Shiranaga, T Mashita, et al.: A hematological study on thirteen cats with myelodysplastic syndrome. *J Vet Med Sci.* **62**, 2000, 59–64.
- RJ Slappendel: Hereditary spherocytosis associated with spectrin deficiency in golden retrievers (abstract). *Proc Eur Soc Vet Intern Med.* 1998, 131.
- JL Spivak: Iron and the anemia of chronic disease. *Oncology.* **16**, 2002, 25–33.
- SL Stockham: Anemia associated with bacterial and viral infections. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 163–168.
- SL Stockham, AM Kjemtrup, PA Conrad, et al.: Theileriosis in a Missouri beef herd caused by *Theileria buffeli*: Case report, herd investigation, ultrastructure, phylogenetic analysis, and experimental transmission. *Vet Pathol.* **37**, 2000, 11–21.
- T Stokol, JT Blue: Pure red cell aplasia in cats: 9 cases (1989-1997). *J Am Vet Med Assoc.* **214**, 1999, 75–79.
- T Stokol, JT Blue, TW French: Idiopathic pure red cell aplasia and nonregenerative immune-mediated anemia in dogs: 43 cases (1988-1999). *J Am Vet Med Assoc.* **216**, 2000, 1429–1436.
- T Stokol, JF Randolph, S Nachbar, et al.: Development of bone marrow toxicosis after albendazole administration in a dog and cat. *J Am Vet Med Assoc.* **210**, 1997, 1753–1756.
- S Tartakover-Matalon, H Shoham-Kessary, V Foltyn, et al.: Receptors involved in the phagocytosis of senescent and diamide-oxidized human RBCs. *Transfusion.* **40**, 2000, 1494–1502.
- NS Taylor, N Dhupa: Acetaminophen toxicity in cats and dogs. *Comp Contin Educ Pract Vet.* **22**, 2000, 160–164.
- V Terpstra, van Berkel, TJC: Scavenger receptors on liver Kupffer cells mediate the in vivo uptake of oxidatively damaged red cells in mice. *Blood.* **95**, 2000, 2157–2163.
- HL Thomas, MA Livesey: Immune-mediated hemolytic anemia associated with trimethoprim-sulphamethoxazole administration in a horse. *Can Vet J.* **39**, 1998, 171–173.
- RE Toribio, FT Bain, DR Mrad, et al.: Congenital defects in newborn foals of mares treated for equine protozoal myeloencephalitis during pregnancy. *J Am Vet Med Assoc.* **212**, 1998, 697–701.
- H Tvedten, DJ Weiss: Classification and laboratory evaluation of anemia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 143–150.
- HW Tvedten: Morphologic classification of anemia. *Vet Clin Pathol.* **28**, 1999, 80–82.
- JN Umbreit, ME Conrad, MA Berry, et al.: The alternate iron transport pathway: Mobilferrin and integrin in reticulocytes. *Br J Haematol.* **96**, 1997, 521–529.
- LM Vap, MA Thrall, MG Weiser: Artifactual macrocytosis in dogs and cats (abstract). *Vet Pathol.* **38**, 2001, 572.
- EJ Villiers, JK Dunn: Clinicopathological features of seven cases of canine myelofibrosis and the possible relationship between the histological findings and prognosis. *Vet Rec.* **145**, 1999, 222–228.
- RM Walton, DE Brown, DW Hamar, et al.: Mechanisms of echinocytosis induced by *Crotalus atrox* venom. *Vet Pathol.* **34**, 1997, 442–449.
- Watson, ADJ: Erythrocytosis and polycythemia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 217–221.
- DJ Weiss, OA Evanson: A retrospective study of feline pancytopenia. *Comp Haematol Int.* **10**, 2000, 50–55.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

DJ Weiss, J Lulich: Myelodysplastic syndrome with sideroblastic differentiation in a dog. *Vet Clin Pathol.* **28**, 1999, 59–63.

DJ Weiss, A Moritz: Equine immune-mediated hemolytic anemia associated with *Clostridium perfringens* infection. *Vet Clin Pathol.* **32**, 2003, 22–26.

DJ Weiss, SA Smith: A retrospective study of 19 cases of canine myelofibrosis. *J Vet Intern Med.* **16**, 2002, 174–178.

JC Whittemore, CA Preston, AE Kyles, et al.: Nontraumatic rupture of an adrenal gland tumor causing intra-abdominal or retroperitoneal hemorrhage in four dogs. *J Am Vet Med Assoc.* **219**, 2001, 329–333, 324.

G Zuniga Gonzalez, MP Ramirez Munoz, O Torres Bugarin, et al.: Induction of micronuclei in the domestic cat (*Felis domesticus*) peripheral blood by colchicine and cytosine-arabioside. *Mutat Res.* **413**, 1998, 187–189.

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## 6 Chapter 5 Evaluation of Leukocytic Disorders

### 6.1 NORMAL LEUKOCYTES

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#### 6.1.1 Classification and Numbers in Blood

Mammalian leukocytes or white blood cells have been classified as either polymorphonuclear or mononuclear leukocytes. Polymorphonuclear leukocytes have condensed, segmented nuclei. They are commonly referred to as *granulocytes* because they contain large numbers of cytoplasmic granules. The term *granulocyte* is preferred in veterinary medicine because nuclear segmentation does not occur in the granulocytes of most reptiles, and it is not as prominent in birds as it is in mammals. The granules in these cells are lysosomes containing hydrolytic enzymes, antibacterial agents, and other compounds. Primary granules are synthesized in the cytoplasm of late myeloblasts or early promyelocytes. They appear reddish purple when stained with routine blood stains such as Wright-Giemsa stain. Secondary (specific) granules appear at the myelocyte stage of development in the bone marrow. Three types of granulocytes (neutrophils, eosinophils, basophils) are identified by the staining characteristics of their secondary granules (Plates 9 to 1011).

In most mammalian species, neutrophil granules either do not stain or appear light pink with routine blood stains. In birds, reptiles, and some mammalian species (e.g., rabbits, guinea pigs, and manatees), the granules of these cells stain red, and the cells are called *heterophils*. They must be differentiated from eosinophils, which also have red-staining granules. The granule shape can often help differentiate these cells. Heterophils usually have rod-shaped or oval granules and eosinophils usually have round granules. Eosinophils are so named because their granules have an affinity for eosin, the red dye in routine blood stains (Plates 9 to 1011). In contrast to round eosinophil granules in most animals, eosinophils from domestic cats have rod-shaped granules. Eosinophils from dogs often exhibit cytoplasmic vacuoles. Eosinophils from greyhounds appear highly vacuolated (Plate 12) and may be mistaken for vacuolated neutrophils by inexperienced observers. Iguanas and psittacine birds have leukocytes with green-staining granules that are believed to be eosinophils. Basophil granules are acidic and consequently have an affinity for the basic (blue) dyes in routine blood stains (Plates 10 and 11). The basophils of domestic cats are distinctive. Their primary granules are dark blue and their secondary granules are light lavender. Often, only the light lavender granules are observed in basophils present in blood smears. The presence of these pale-staining granules overlying the nucleus gives the nucleus a moth-eaten appearance, and this nuclear characteristic, along with overall cell sizes larger than neutrophils, helps observers identify these cells at low magnifications.

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Mononuclear leukocytes in blood are classified as either lymphocytes or monocytes (Plates 13 and 15). These cells are not devoid of granules, but rather have lower numbers of cytoplasmic granules than do granulocytes. T and B lymphocytes cannot be differentiated from one another on the basis of morphology on stained blood films. Lymphocytes have high nuclear to cytoplasmic ratios. Their nuclei have coarsely clumped chromatin. Nuclei are usually round but may be oval or slightly indented. Most lymphocytes in the blood of domestic animals are small to medium in size. Lymphocytes in cattle are often larger with more abundant cytoplasm than is seen in other species, sometimes making these cells difficult to differentiate from monocytes (Plate 15). Monocytes are usually larger than lymphocytes, have nuclei with finer chromatin clumping that are more variable in shape (round-, kidney-, or band-shaped), and have nuclear to cytoplasmic ratios of 1 or less. Monocytes often exhibit cytoplasmic vacuoles on films prepared from blood collected with EDTA as the anticoagulant. A few lymphocytes in blood have focal accumulations of red- or purple-staining granules within the cytoplasm (Plate 17). These granular lymphocytes may be cytotoxic T lymphocytes or natural killer (NK) cells. Dust-like red- or purple-staining granules may also be seen dispersed within the cytoplasm of monocytes.

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The total number of leukocytes varies considerably by species. Among common domestic animals, the mean total leukocyte count is highest in pigs (16,000/ $\mu$ L) and lowest in cattle and sheep (8000/ $\mu$ L). Neutrophils and lymphocytes are the most numerous leukocyte types present in blood of healthy domestic mammals. Dogs, cats, and horses usually have more neutrophils than lymphocytes in blood. In contrast, lymphocytes are usually more numerous in the blood of pigs, cattle, sheep, and goats. Numbers of neutrophils and lymphocytes change with age after birth. The neutrophil to lymphocyte ratio tends to be higher at birth than in later life, in part because of the increased blood cortisol concentration at birth. Cortisol causes the number of circulating neutrophils to increase and the number of circulating lymphocytes to decrease. Ruminants have neutrophil to lymphocyte ratios above 1.0 at birth because they have more circulating neutrophils and fewer circulating lymphocytes than adults. Within 24 hours, neutrophil numbers decrease and lymphocyte numbers increase, with lymphocytes exceeding neutrophils during the first week of life. Low numbers of monocytes, eosinophils, and basophils are present in healthy mammals. Basophil numbers are especially low in dogs and cats, with none being seen in blood from many healthy animals.

6.1.2

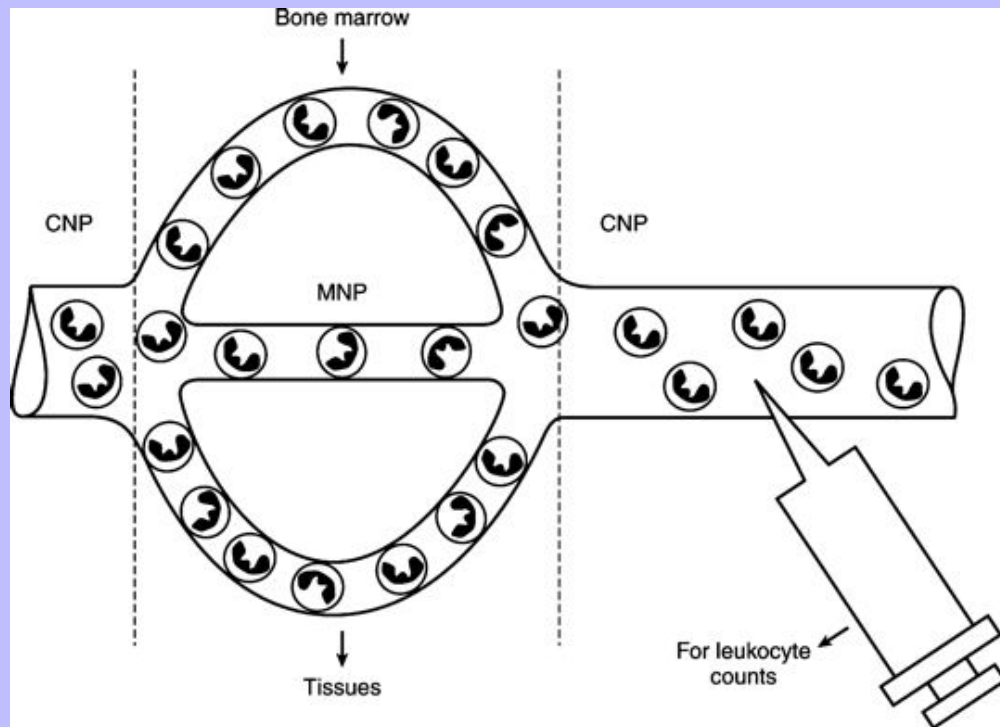
## Leukocyte Kinetics

In contrast to erythrocytes, leukocytes do not exhibit a life span in blood, but rather leave blood at random times in response to chemoattractant stimuli. After release from bone marrow, neutrophils are normally present in blood for only a few hours before egress into the tissues. Neutrophils that remain in blood spontaneously undergo apoptosis and are removed by macrophages (e.g., Kupffer's cells in the liver). Neutrophils occur in circulating and marginating pools in blood, with 50% or less of the total blood neutrophil pool being present in the circulating pool (Fig. 5-1). The circulating neutrophil pool (CNP) is assessed by routine blood sample collection. The marginating neutrophil pool (MNP) consists of neutrophils that are transiently retained in capillaries, especially those in the lungs. This retention of neutrophils in capillaries does not appear to involve neutrophil adhesion to endothelial cells. Rather, retention appears to be a mechanical process, resulting from neutrophil stiffness in contrast to the ready deformability of erythrocytes. The absolute neutrophil count measured in blood samples can be affected by cell movements between the MNP and the CNP. A net movement of neutrophils from the MNP to the CNP increases the circulating blood neutrophil count. A net movement in the opposite direction results in a decreased circulating blood neutrophil count. Eosinophils, basophils, and monocytes are also present in blood for a short time (less than a day) before they leave the blood and enter the tissues at random times.

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Fig. 5-1



Neutrophil distribution in blood. Neutrophils occur in the circulating neutrophil pool (CNP) and marginating neutrophil pool (MNP), with 50% or less of the total blood neutrophil pool being present in the CNP.

Except for certain lymphocyte populations, leukocytes do not reenter the circulation after migration into the tissues. Neutrophils, eosinophils, and basophils appear to survive no more than a few days in tissues, and their survival is much shorter in sites of inflammation. In contrast, monocytes develop into macrophages or dendritic cells in the tissues, where they survive for weeks to months under normal conditions.

Most lymphocytes reside within lymphoid organs (lymph nodes, thymus, spleen, and bone marrow). A small number of lymphocytes circulate in blood. Most lymphocytes in blood have come from peripheral lymphoid organs (primarily lymph nodes). Depending on the species and individual variability, about 50% to 75% of blood lymphocytes are T lymphocytes and about 10% to 40% are B lymphocytes. NK cells appear as granular lymphocytes in most species and account for 5% to 10% of blood lymphocytes. Many blood lymphocytes are memory cells, which are thought to have been antigen-primed and to exist in a resting state. They naturally express levels of adhesion molecules that allow them to circulate from blood, through tissue, and then back into blood. It is estimated that recirculating lymphocytes enter a new lymphoid organ or tissue every 1 to 2 days. This allows the full repertoire of lymphocytes to be available for immune reactions throughout the body. Most of the blood lymphocytes migrate into lymph nodes through high endothelial venules and exit the lymph nodes in the efferent lymphatics. Efferent lymphatics join to form large lymphatic vessels, the largest of which is the thoracic duct, and drain into the blood at the level of the heart (Fig. 5-2). Blood lymphocytes also enter tissues

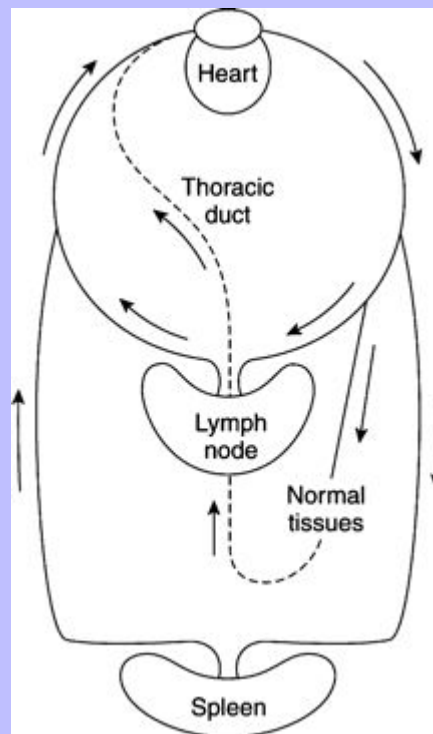
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such as skin, intestinal mucosa, and lungs through vessels with activated endothelial cells. Tissue lymphocytes are picked up by peripheral lymphatics and carried to lymph nodes where they exit by efferent lymphatics, traverse large lymphatic vessels, and reenter the blood. Recirculating lymphocytes bind to and traverse high endothelial venules and vessels with activated endothelial cells because these cell types have adhesion molecules on their surfaces that recognize complementary adhesion molecules expressed on lymphocyte surfaces. Lymphocytes that have been exposed to an antigen in a given tissue tend to develop adhesion molecules that favor their return to that tissue. Most plasma cells and proliferating lymphocytes, such as those present in germinal centers, do not express the adhesion molecules necessary for migration.

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Fig. 5-2



Circulation routes for lymphocytes. Solid lines represent blood vessels, and dashed lines represent lymphatic vessels.

Lymphocytes generally survive much longer than granulocytes. T lymphocytes generally survive several years. Memory B lymphocytes are also long-lived. Survival of other B lymphocytes is shorter, often lasting only a few days. NK cell life spans have not been well characterized, with values from a few days to several months being reported in laboratory animals.

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## 6.1.3 Leukocyte Functions

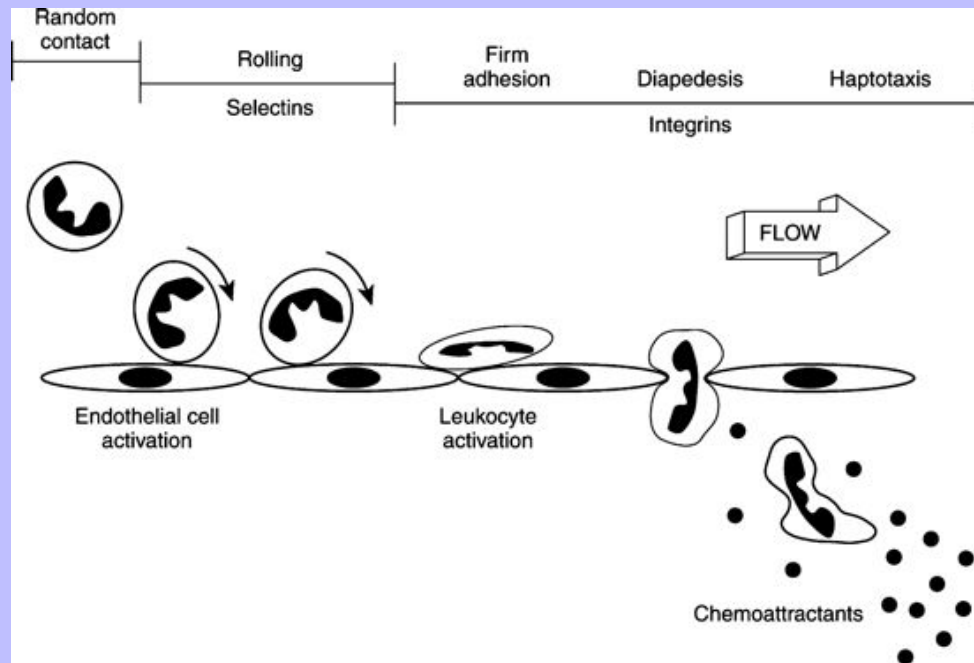
### 6.1.3.1 Neutrophil Functions

Neutrophils are essential in the defense against invading microorganisms, primarily bacteria. To be effective, they must recognize inflammatory signals, leave the blood, migrate through tissue to a site where bacteria are present, and then neutralize the bacteria. Neutrophils display glycoprotein adhesion molecules on their surfaces that are needed for various adhesion-dependent functions including adhesion to endothelium and subendothelial structures, spreading, haptotaxis, and phagocytosis. Unless activated, neutrophils and endothelial cells exhibit little tendency to adhere to each other. When endothelial cells are activated by inflammatory mediators including interleukin-1 (IL-1) and tumor necrosis factor (TNF), they rapidly express P-selectin (from storage granules) and E-selectin adhesion molecules on their surfaces. The expression of these oligosaccharide-binding glycoproteins, acting in concert with the L-selectin adhesion molecule expressed on the surfaces of neutrophils, results in the initial adhesion of unstimulated neutrophils to activated endothelial cells (Fig. 5-3). As a result, the velocity of neutrophils in the circulation is markedly decreased, and they are seen to roll along endothelium.

Activated endothelial cells produce factors including IL-8 and platelet-activating factor (a biologically active phospholipid) that result in neutrophil activation. Other mediators that can activate neutrophils include opsonized particles, immune complexes, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and chemoattractants produced during inflammation. Neutrophil activation results in increased expression and enhanced binding affinity of  $\beta_2$  integrin adhesion molecules, but shedding of L-selectin molecules.  $\beta_2$  Integrins (CD11a,b,c/CD18) are heterodimers that bind with variable affinity to intercellular adhesion molecules. The tight binding of  $\beta_2$  integrins to intercellular adhesion molecules (which are up-regulated on activated endothelial cells) results in firm adhesion of neutrophils to endothelial cells. Adherent (activated) neutrophils spread and exhibit pseudopod formation. Neutrophil activation also promotes degranulation, superoxide generation, and production of arachidonate metabolites to be discussed later.

In addition to increased affinity for  $\beta_2$  integrins, activated neutrophils have increased numbers of surface receptors and enhanced receptor affinity for chemoattractants. These receptors are also found in granules, suggesting that they are mobilized to the cell surface during neutrophil activation. When exposed to a chemoattractant, neutrophils penetrate the vessel wall by moving between endothelial cells. Neutrophils crawl toward the source of the chemoattractant in a “treadmill” pattern of motion by apposition of  $\beta_2$  integrin adhesion molecules (in concert with  $\beta_1$  and  $\beta_3$  integrin molecules) to their respective receptors on cells or within the extracellular matrix. The active directional migration of neutrophils in tissues occurs by haptotaxis, which is migration up a gradient of immobilized chemoattractants, rather than soluble chemoattractants (chemotaxis). A wide variety of substances can function as chemoattractants. They include IL-8 and other chemokines, C5a (complement fragment), leukotriene B<sub>4</sub> (a product of arachidonic acid metabolism via the lipoxygenase pathway), platelet-activating factor (1-0-alkyl-2-acetyl *sn*-glyceryl phosphoryl choline), and bacterial products (e.g., N-formyl-methionyl oligopeptides).

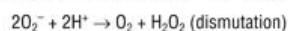
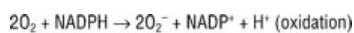
Fig. 5-3



Endothelial cell activation, neutrophil rolling along vessel walls, tight adhesion between neutrophils and endothelial cells, diapedesis, and haptotaxis.

For phagocytosis to occur, neutrophils must be able to first bind invading bacteria to their surfaces (Fig. 5-4). This adherence is greatly potentiated if bacteria have been opsonized (have antibodies and complement components bound to their surfaces) because neutrophils have immunoglobulin Fc and C3b receptors on their surfaces. After binding, bacteria are engulfed by neutrophil cytoplasmic processes, which extend around the organisms. The membranes of the neutrophil cytoplasmic processes fuse to form a phagocytic vacuole surrounding the engulfed bacteria.

Bacterial killing involves a multiplicity of mechanisms that are set into motion by two cellular events, initiation of the respiratory burst and degranulation. The respiratory burst is initiated by the activation of a reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme (Fig. 5-5). This enzyme is normally “inactive” in resting (unstimulated) phagocytes. Enzyme activation depends on the assembly of multiple components, some of which are already membrane-bound and others that must be translocated from the cytoplasm to the membrane. Activated NADPH oxidase is located in the plasma membrane and becomes incorporated into the phagocytic vacuole. It catalyzes the one-step reduction of oxygen ( $O_2$ ) to form superoxide ( $O_2^-$ ). The NADPH needed to generate superoxide is formed in the pentose phosphate pathway. The superoxide formed undergoes dismutation to form hydrogen peroxide as shown in the following equations.



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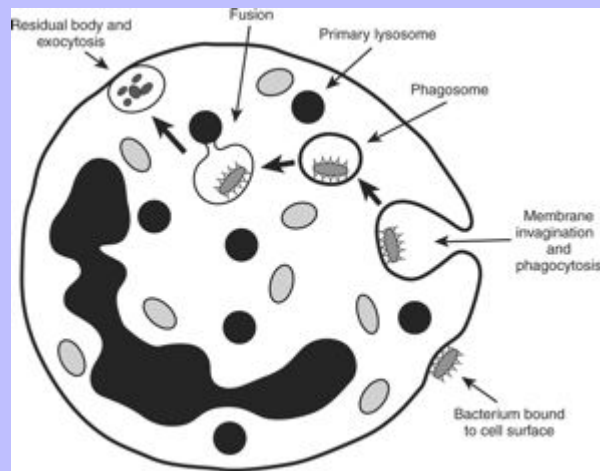
Hydrogen peroxide and superoxide can diffuse from the phagocytic vacuole into the cytoplasm of the cell.

Activated neutrophils utilize the superoxide dismutase and glutathione peroxidase reactions to protect 86

themselves from these oxidants. The latter reaction requires that additional NADPH be generated to maintain 87

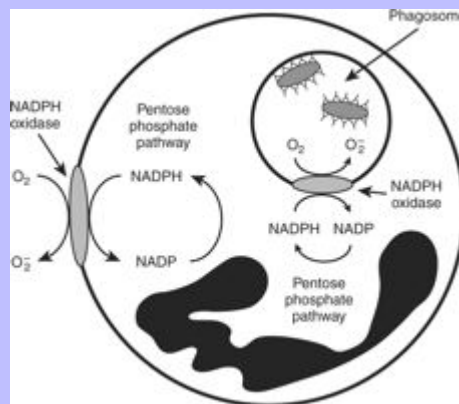
glutathione in the reduced form. Activation of the respiratory burst requires neither phagocytosis nor degranulation to occur. In addition to opsonized particles, soluble factors, such as C5a, can activate the respiratory burst.

Fig. 5-4



Basic events involved in the phagocytosis, killing, and discharge of killed bacteria and degraded bacterial products.

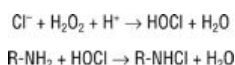
Fig. 5-5



Generation of superoxide free radicals by the membrane-associated reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme. *NADP*, Nicotinamide adenine dinucleotide phosphate.

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Superoxide, other free radicals (e.g., hydroxyl radical), and  $\text{H}_2\text{O}_2$  may be involved directly in the killing of bacteria; but killing is potentiated by degranulation, which results in the fusion and release of contents of lysosomal granules into the phagocytic vacuole (see Fig. 5-4). Myeloperoxidase is an iron-containing enzyme located in the primary granules of neutrophils. The myeloperoxidase reaction greatly enhances the bactericidal potency of  $\text{H}_2\text{O}_2$ . This reaction apparently catalyzes oxidation of chloride to hypochlorous acid, resulting in halogenation of bacterial cell walls (see the following equations) and loss of integrity.



Other enzymes are also present in primary and secondary granules. These include collagenase; acid and neutral hydrolases; and lysozyme, which hydrolyzes glycosidic linkages in cell walls of certain bacteria. These enzymes are probably more important in digestion than in killing. Nonenzymatic agents are also involved in neutrophil defense. A number of cationic proteins and peptides in neutrophil granules have antimicrobial properties. Of these molecules, defensins appear to be the most common. With the lowest molecular weight, defensins are small antimicrobial peptides (4 kd) within primary granules that act against bacteria and other microorganisms by altering their membrane permeability. They are inserted into the lipid bilayer, disrupting interaction between lipid molecules. Lactoferrin occurs within secondary granules. It chelates iron required for microbial growth.

After killing and digestion of bacteria, the phagocytic vacuole fuses with the plasma membrane and discharges killed bacteria, products of degraded bacteria, and contents of granules to the outside of the cell in a process called *exocytosis* (see Fig. 5-4). Discharge of granules can also occur after activation of neutrophils in the absence of phagocytosis. Considerable tissue injury occurs in areas in which neutrophils are activated because of the oxidants they produce and the granule contents they release.

### 6.1.3.2

#### Eosinophil Functions

Functions of eosinophils are not completely defined. They have limited phagocytic abilities and provide poor host defense against bacterial or viral agents. Eosinophils are active in killing parasites, such as flukes and helminths in the tissue stages, that have antibodies and/or complement bound to their surfaces. Eosinophils release substances that inhibit some of the inflammatory effects of mast cell degranulation. However, activated eosinophils also generate inflammatory mediators, which can result in tissue injury.

Flukes and helminths stimulate both humoral and cellular immunity. B lymphocytes produce immunoglobulin G (IgG) antibodies that may bind to parasites and activate complement, thereby inflicting damage to the parasite and initiating an inflammatory reaction. Specific IgE antibodies that bind to mast cells may also be produced. The binding of parasite antigens to these antibodies results in mast cell activation and degranulation and release of inflammatory mediators and potent chemoattractants for eosinophils. These chemoattractants include histamine, leukotriene C4 (a product of the lipoxygenase pathway of arachidonic acid metabolism), and small to intermediate size peptides. C-C chemokine peptides, such as eotaxin and RANTES, appear to be particularly important in the selective recruitment of eosinophils. Other factors, such as the C5a component of complement, platelet-activating factor, and leukotriene B4, also function as chemoattractants, but these factors are not specific for eosinophils. T lymphocytes activated by parasite antigens produce factors (IL-5, IL-3, and GM-CSF) that not only stimulate the production and release of eosinophils but also activate eosinophils and promote their survival.

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Similar to neutrophil adhesive processes, selectins and  $\beta_2$  integrins are involved in eosinophil adhesion to activated endothelial cells. In addition, endothelial cells activated by IL-4 express vascular cell adhesion molecule-1 (VCAM-1), which binds to very late antigen-4 (VLA-4) on the surface of eosinophils. This integrin is not expressed on neutrophils and presumably helps provide specificity for eosinophil localization.

Eosinophils accumulate in the tissues in response to chemoattractants, which are generated in response to parasites. They bind to the opsonized parasites via their surface receptors to IgG and complement. The parasites are much too large for eosinophils to ingest, but when activated, eosinophils exhibit dramatic NADPH oxidase activity, which generates extracellular oxidants. They also exocytose their granules in the area of the invading parasite. Eosinophil peroxidase released from granules interacts with hydrogen peroxide generated from the respiratory burst and halide ions. This complex, along with other oxygen metabolites and major basic protein released from secondary granules, is primarily involved in the killing of helminths and flukes. 88 89

### 6.1.3.3

#### Basophil Functions

Basophils generally occur in low numbers in the circulation. They contain most of the histamine measured in blood. Histamine in granules is bound to polyanions (including heparin) and these polyanions are responsible for the metachromatic staining (purple color with blue dyes) of the granules. Basophils have biochemical characteristics similar to those of mast cells and probably share a common progenitor cell with mast cells in bone marrow, but they are clearly different cell types. Basophils have segmented nuclei and mast cells have round nuclei. Mast cells usually have more cytoplasmic granules than basophils. In cats, both primary and secondary granules in basophils are morphologically different from mast cell granules.

The functions of basophils are unclear, but they may be similar to mast cells once the basophils migrate into tissues. As discussed previously, mast cell activation and degranulation result in inflammation that may help expel metazoan parasites and recruit eosinophils that kill these parasites. Basophils and mast cells are involved in allergic conditions. After binding of an antigen to a specific surface-bound IgE antibody, these cells degranulate and release histamine and other mediators that account for the inflammation present in immediate hypersensitivity reactions. Other foreign material (physical or chemical agents) can also cause degranulation of these cells. In some instances, this reaction may help expel the foreign material.

### 6.1.3.4

#### Monocyte/Macrophage Functions

Macrophage colony-stimulating factor (M-CSF) not only stimulates monocyte production but also stimulates the transformation of monocytes into macrophages. Although most tissue macrophages are of blood monocyte origin, some proliferation of macrophages can be stimulated by growth factors in tissues during inflammation. The development of monocytes into macrophages is associated with a fivefold to tenfold increase in size, an increase in granules (lysosomes), an increase in size and number of mitochondria, and an increase in phagocytic capacity. Macrophage function is augmented by various cytokines, the most potent of which is reported to be  $\gamma$ -interferon. M-CSF and GM-CSF also enhance macrophage function. The mononuclear phagocyte system consists of “fixed” macrophages (Kupffer's cells in the liver, littoral cells in the spleen, and nurse cells in marrow), mobile phagocytic cells (monocytes, peritoneal and pleural macrophages, and alveolar macrophages), and multinucleated giant cells, which may form during chronic inflammatory conditions from a fusion of other mononuclear phagocytes.

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Macrophages move more slowly and are less potent in killing bacteria but are notably more active in fungal and viral infections than are neutrophils. Macrophages can synthesize new membrane material and replace expended lysosomes. Therefore they have more staying power in combating infections than do neutrophils, which have limited synthetic abilities.

Antimicrobial properties of macrophages are less well understood than those of neutrophils. Lactoferrin is absent, but NADPH oxidase and myeloperoxidase activities are present, although the activity of the latter is considerably less than that observed in neutrophils. Lysozyme is present, but few organisms are sensitive to it in their native states. Nitric oxide, a free radical generated from L-arginine, appears to be important in microbial killing by macrophages, especially after nitric oxide interacts with superoxide to generate the toxic derivatives peroxynitrite and the nitrogen dioxide radical.

Macrophages demonstrate necrotaxis and necrophagocytosis (phagocytosis of devitalized tissue). Opsonization of necrotic tissue is not required for necrophagocytosis to occur. Consequently, macrophages serve an important function in cleaning up necrotic tissue and other debris within the body.

Macrophages have other important functions in immunity, including antigen processing; killing of tumor cells after sensitization by T lymphocytes; and synthesis of CSFs, interleukins, complement components, interferon, and TNF. Macrophages remove aged or damaged erythrocytes and store the iron from these cells in the form of ferritin and hemosiderin. Macrophages are also necessary for normal wound healing.

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Macrophages in the liver and spleen are most important in clearing blood-borne pathogens of dogs, rodents, rabbits, monkeys, and humans, but intravenous pulmonary macrophages are most important in defending against blood-borne pathogens in cats, ruminants, pigs, and horses.

## 6.1.3.5

### Lymphocyte and Natural Killer Cell Functions

A thorough discussion of lymphocyte and NK cell functions is beyond the scope of this text; consequently, the reader is referred to current immunology textbooks such as the one by Tizard (2000) for more detailed information. The production and location of lymphocyte types are discussed in [Chapter 3](#). Although bone marrow and thymus seed other lymphoid organs with B and T lymphocytes, respectively, it should be noted that local proliferation of lymphocytes occurs in lymphoid organs in response to specific antigens.

B lymphocytes are primarily responsible for humoral immunity; however, immunoglobulin production also requires the participation of T lymphocytes, dendritic cells, and macrophages. Dendritic cells and macrophages bind foreign antigens and process them so that they become highly immunogenic. A processed antigen is presented to virgin T lymphocytes and B lymphocytes. Interactions between T lymphocytes, B lymphocytes, and the processed antigen result in a clonal proliferation of both cell types. When these antigen-specific cells recognize the same antigen at a later date, they undergo a secondary heightened proliferative response. Antigen activation of B lymphocytes may also occur independently of T lymphocytes, if the antigen is a high-molecular-weight polymer. After activation, B lymphocytes are transformed into immunoglobulin-producing immunocytes and plasma cells. IgM is initially produced by these cells, but with continued antigenic stimulation, IgG becomes the predominant antibody type produced. Clonal amplification of these cells results in the production of greater amounts of antibody against the foreign antigen. In addition to immunoglobulin production, B lymphocytes produce cytokines that may influence the proliferation and/or function of other blood cell types.

T lymphocytes are largely responsible for cellular immunity. In contrast to B lymphocytes, which produce immunoglobulins that are carried to the site of a foreign antigen, T lymphocytes can migrate to the site of a

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foreign antigen. T lymphocytes are involved in immune regulation, cytotoxicity, delayed-type hypersensitivity, and graft-versus-host reactions. They are also actively involved in the control of hematopoiesis. Different subpopulations produce a large number of cytokines with diverse biologic activities to achieve these effects. Normal immune regulation requires a balanced participation of T-helper and T-suppressor lymphocytes. Altered T-lymphocyte function may result in the development of autoimmune diseases. Cytotoxic T lymphocytes are antigen-dependent cells that can destroy target cells (e.g., neoplastic cells) by a contact-dependent, major histocompatibility complex-dependent, nonphagocytic process.

NK cells appear as granular lymphocytes in most species. These cytotoxic cells lyse target cells without deliberate prior sensitization and without restriction by major histocompatibility complex antigens.

## 6.2 ABNORMAL NEUTROPHIL MORPHOLOGY

### 6.2.1 Toxic Cytoplasm

The cytoplasm of neutrophils from common domestic animals is nearly colorless. When increased basophilia, foamy vacuolation, and/or Döhle bodies are seen in the cytoplasm of a neutrophilic cell, the cell is said to be toxic (Plate 14). These morphologic abnormalities in the cytoplasm represent maturational defects that form in the bone marrow during “toxic” conditions. Toxic cytoplasm is primarily seen in association with strong inflammatory conditions, and severe bacterial infections are the most common cause.

#### 6.2.1.1 Foamy Basophilia

Foamy basophilia often occurs with severe bacterial infections but can occur with other causes of toxemia. When viewed by means of electron microscopy, foamy vacuolation appears as irregular, electron-lucent areas that are not membrane bound. Cytoplasmic basophilia results from the persistence of large amounts of rough endoplasmic reticulum and polyribosomes.

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#### 6.2.1.2 Döhle Bodies

Döhle bodies are bluish angular cytoplasmic inclusions of neutrophils composed of retained aggregates of rough endoplasmic reticulum. By themselves, these inclusions represent mild evidence of toxicity and are sometimes seen in neutrophils of cats that do not exhibit signs of illness. Döhle bodies must be differentiated from iron-positive granules, distemper inclusions in dogs, and granules present in neutrophils from cats with inherited Chédiak-Higashi syndrome.

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#### 6.2.1.3 Toxic Granulation

Toxic granulation refers to the presence of magenta-staining cytoplasmic granules. These granules consist of primary granules that have retained the staining intensity normally observed in promyelocytes in the bone marrow. The presence of toxic granulation and cytoplasmic basophilia suggests severe toxemia. Toxic granulation may be seen in horses, cattle, and sheep; but it is rarely seen in dogs and cats. Toxic granulation should not be confused with the pink staining of secondary granules, which is not a sign of toxicity. Purple granules are often seen in neutrophils from foals without other evidence of cytoplasmic toxicity; however, the clinical significance of this is unclear. Toxic granulation must be differentiated from the granules present in some apparently healthy cats and in animals with certain lysosomal storage disorders.

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### 6.2.2 Inherited Disorders

Chédiak-Higashi syndrome is a disorder characterized by enlarged cytoplasmic granules in many types of granule-containing cells. Neutrophils from affected cats and cattle contain large pink to red granules. Small reddish granules have been reported as an inherited anomaly in Birman cats without evidence of illness. Similarly, persistent reddish granulation has been seen in neutrophils of several apparently healthy Siamese and Himalayan cats. Basophilic granulation occurs in the cytoplasm of neutrophils from cats and dogs with certain lysosomal storage disorders including mucopolysaccharidosis VI, mucopolysaccharidosis VII, and GM<sub>2</sub> gangliosidosis.

### 6.2.3 Infectious Agents

Distemper inclusions are formed in bone marrow precursor cells and may be present in blood cells during the acute viremic stage of the disease. These viral inclusions can be difficult to visualize in the cytoplasm of neutrophils on Wright- or Giemsa-stained blood films but can easily be seen as homogeneous round, oval, or irregularly shaped 1- to 4-μm red inclusions, when stained with Diff-Quik (Plate 8). Morulae of *Ehrlichia* and *Anaplasma* species that infect neutrophils (e.g., *Ehrlichia ewingii* and *Anaplasma phagocytophilum*) may be found in neutrophils during the acute stage of infection (Fig. 5-6). These morulae appear as tightly packed basophilic clusters of organisms within the cytoplasm. Gametocytes of the protozoal organism *Hepatozoon americanum* may be seen in the cytoplasm of circulating neutrophils. These appear as large oblong structures. The nucleus of a gametocyte usually stains poorly with routine blood stains.

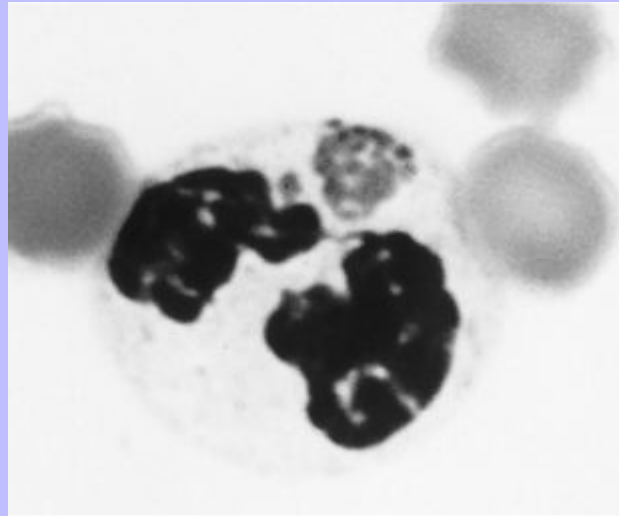
### 6.2.4 Left Shifts

When increased numbers of immature neutrophils are present in blood, their presence is referred to as a *left shift* (Plate 14). Left shifts are usually associated with inflammatory conditions. These inflammatory conditions are often infectious but may be noninfectious (e.g., immune-mediated disorders and infiltrative marrow disease). The presence of a significant left shift in an animal with an inflammatory disorder indicates that the stimulus for release of neutrophils from bone marrow is greater than can be accommodated by release from mature neutrophil stores alone. The magnitude of a left shift in response to inflammation can vary from slightly increased numbers of bands to severe left shifts with metamyelocytes, myelocytes, and—rarely—even promyelocytes present in blood. The total neutrophil count may be low, normal, or high, depending on the number of these cells released from the bone marrow versus the number utilized in the inflammatory process. Toxic cytoplasm is often present in animals with left shifts in response to inflammatory disorders.

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Fig. 5-6



Horse blood neutrophil containing an *Anaplasma phagocytophilum* morulae. (Wright-Giemsa stain.)

The term *regenerative left shift* is used when neutrophilia with a left shift is present and the mature neutrophils predominate. The term *degenerative left shift* is used when the immature neutrophils outnumber mature neutrophils and the total neutrophil count is normal or low.

The term *hyposegmentation* refers to a left shift that is due to the presence of neutrophils that have the condensed nuclear chromatin of mature neutrophils but lack nuclear constrictions normally present in mature neutrophils. Hyposegmentation occurs as an inherited trait in the Pelger-Huët anomaly in dogs and cats. Eosinophils and basophils may also be affected. No clinical signs are associated with animals that are heterozygous for this disorder. Homozygous affected animals die in utero. The Pelger-Huët anomaly is common in Australian shepherd dogs, in which it appears to be transmitted as an autosomal dominant trait with incomplete or decreased penetrance. A pseudo-Pelger-Huët anomaly may occur transiently with chronic infections or, rarely, with the administration of certain drugs.

### 6.2.5 Hypersegmentation

Hypersegmentation (right shift) refers to the presence of five or more distinct nuclear lobes within neutrophils. It occurs as a normal aging process and may reflect prolonged transit time in blood, as can occur with resolving chronic inflammation, with glucocorticoid administration, or with hyperadrenocorticism. It may develop in vitro when blood film preparation is delayed more than a few hours. Hypersegmentation may be present in myeloproliferative disorders. Idiopathic hypersegmentation has been reported in horses without evidence of clinical disease. It has also been described in dogs with an inherited defect in cobalamin absorption and in a cat with folate deficiency.

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## 6.3 NEUTROPHILIA

Neutrophilia may develop as a result of increased neutrophil production and/or release from the bone marrow, decreased movement of neutrophils from blood into the tissues, or net movement of neutrophils from the MNP to the CNP as shown in Fig. 5-7. Neutrophilia develops rapidly in blood after release of epinephrine, as occurs with exercise, fear, or excitement. This results from a shift of neutrophils from the MNP to the CNP. The cell count usually does not increase above twice the normal value and no left shift occurs (Table 5-1). The epinephrine effect is more commonly seen in young animals. Some animals (e.g., cats and horses) may exhibit an accompanying lymphocytosis. Leukogram effects should return to normal within 30 minutes of removal of the stimulus.

The increased endogenous release or exogenous administration of glucocorticoid steroids has profound effects on circulating blood cell numbers within a few hours after release or administration. Potential causes of increased endogenous release of glucocorticoids include pain, prolonged emotional stress, abnormal body temperature, and hyperadrenocorticism. The duration of effects depends on the nature of the exogenous glucocorticoid administered (long-acting or short-acting). Neutrophilia occurs because glucocorticoids cause increased release of mature neutrophils from bone marrow stores and decreased egress of neutrophils from blood into tissues. A higher proportion of neutrophils is also present in the CNP compared with the MNP, but the size of the MNP may not actually be decreased, because the total blood neutrophil pool is increased. The absolute number of neutrophils seldom increases above twice the normal value, and a left shift is usually not present. Glucocorticoids also cause lymphopenia and eosinopenia in all domestic animals (Table 5-1). Monocytosis is commonly observed in dogs, and occasionally, in cats. The magnitude of the neutrophilia decreases with time, but the lymphopenia and eosinopenia persist as long as plasma glucocorticoid concentrations are increased. For example, most dogs with Cushing's disease have lymphopenia and eosinopenia with normal neutrophil counts.

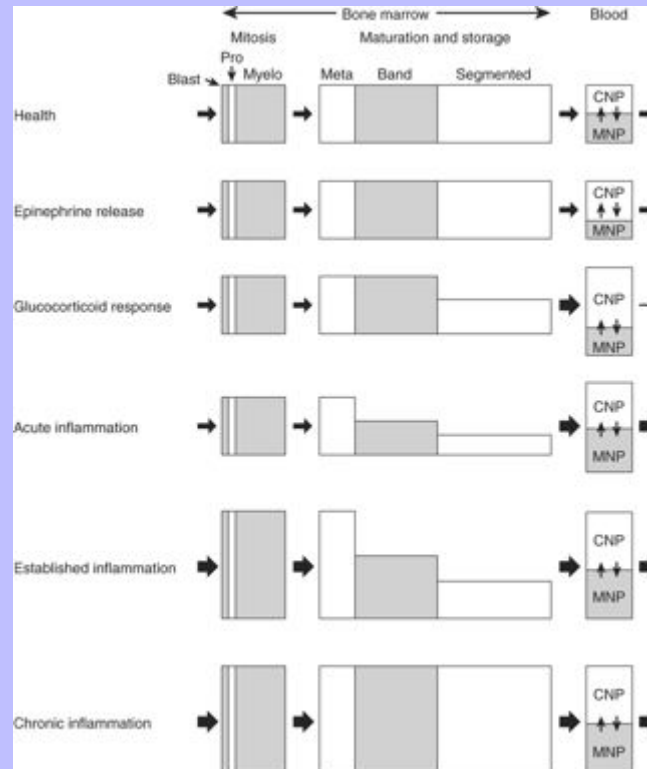
Table 5-1 Expected findings in different types of neutrophilia

Type	Lymphocyte count	Left shift
Physiologic (epinephrine)	Normal or increased	None
Stress (glucocorticoids)	Usually decreased	None or slight
Inflammation	Often decreased	Often present

Neutrophilia without a significant left shift may also be present in association with hemorrhage, hemolysis, necrosis, chemical and drug toxicities, malignancy, mild inflammation, and some chronic inflammatory conditions. The mechanism(s) causing neutrophilia in these disorders is not always clear. Various conditions can result in increased concentrations of hematopoietic growth factors (especially G-CSF) in the circulation, which result in increased neutrophil production and release. The inflammatory cytokines IL-1 and TNF induce neutrophilia by stimulating the production of growth factors such as G-CSF and GM-CSF. In mild inflammatory conditions and some chronic inflammatory conditions, the increased peripheral demand for neutrophils is met by increased production and release of mature neutrophils from the marrow.

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Fig. 5-7



Mechanisms responsible for the production of a neutrophilia. *CNP*, Circulating neutrophil pool; *MNP*, marginating neutrophil pool.

The neutrophil response to inflammatory stimuli is more muted in ruminants than in other domestic animals. Neutrophilia is less common and neutropenia is more common in response to acute bacterial infections in ruminants compared with other species. Total leukocyte counts of  $20 \times 10^3/\mu\text{L}$  to  $30 \times 10^3/\mu\text{L}$  are considered to be markedly elevated in ruminants. Detection of increased concentrations of acute-phase proteins, such as haptoglobin and fibrinogen, can provide evidence of chronic inflammation that may not be seen on the leukogram.

Prominent left shifts are often associated with inflammatory conditions. These may be infectious (bacterial, viral, fungal, protozoal) or noninfectious (immune-mediated) disorders. The presence of a significant left shift indicates that the stimulus for release of neutrophils from bone marrow is greater than can be accommodated by release from mature neutrophil stores alone. Regenerative left shifts are generally viewed as an adequate marrow response at that moment. However, the presence of significant cytoplasmic toxicity requires a guarded prognosis. A marked leukocytosis (total leukocyte count of greater than  $50,000/\mu\text{L}$ ) with neutrophilia and marked left shift back to at least myelocytes associated with an inflammatory condition is called a *leukemoid reaction*, because it resembles the blood pattern seen in chronic myeloid leukemia (CML) (discussed later in this chapter). Left shifts associated with leukemoid reactions are usually orderly, with mature segmented neutrophils being the most numerous neutrophilic cells present, bands being the next most numerous, metamyelocytes being less numerous, and

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myelocytes being present in the lowest numbers. A localized purulent inflammatory condition, such as pyometra, should be suspected when a leukemoid response is present.

Disorders that may stimulate extreme neutrophilic leukocytosis in dogs and cats (leukocyte counts above 50,000/ $\mu$ L with greater than 25,000 neutrophils/ $\mu$ L) include infections (such as pyothorax, pyelonephritis, septic peritonitis, pyometra, abscess, pneumonia, and hepatitis), immune-mediated disorders (such as immune-mediated hemolytic anemia, glomerulonephritis, polyarthritis, and vasculitis), neoplasia (such as lymphoma, acute and chronic myeloid leukemia, and mast cell tumors), and tissue necrosis (caused by trauma and diseases such as pancreatitis, thrombosis, and bile peritonitis). Neutrophilic leukocytosis with leukocyte counts as high as 200,000/ $\mu$ L has been reported in dogs with *Hepatozoon americanum* infection, which causes pyogranulomatous myositis. Extreme leukocytosis also occurs during the first 3 weeks after the injection of a toxic dose of estrogen in dogs. This neutrophilic hyperplastic phase in the marrow is followed by generalized hypoplasia or aplasia and death or slow recovery. Neutrophilia in animals with a wide variety of tumors may result from inflammation or necrosis within the tumor; but neutrophilia may also occur as a paraneoplastic phenomenon caused by the production of growth factors, such as G-CSF and GM-CSF, by the tumor.

CML is a common disorder in humans but is rarely reported in animals. Most cases have been reported in dogs. Animals with CML have persistent marked neutrophilia with a pronounced left shift that may extend to myeloblasts. This diagnosis is usually reached by ruling out inflammatory causes and documenting the concomitant occurrence of additional proliferative abnormalities in blood and bone marrow.

Neutrophilia with or without a modest left shift is present in some animals with inherited neutrophil dysfunctions. Profound neutrophilia occurs in dogs and cattle with  $\beta_2$  integrin adhesion molecule deficiency. Both increased production of neutrophils and decreased egress of neutrophils from blood into the tissues contribute to the high number of neutrophils in blood of animals with this inherited defect. Inherited neutrophil dysfunctions should be included in the differential diagnosis when unexplained, recurrent bacterial infections occur in a young animal.

## 6.4 NEUTROPENIA

Neutropenia can develop from decreased release of neutrophils from bone marrow, increased egress of neutrophils from blood, destruction of neutrophils within the blood, or a shift of neutrophils from the CNP to the MNP (Fig. 5-8). Healthy Belgian Tervuren (a variety of Belgian shepherd) dogs frequently have total leukocyte counts, absolute neutrophil counts, and absolute lymphocyte counts below reference intervals established for dogs; consequently, this finding has been described as a physiologic leukopenia in this breed.

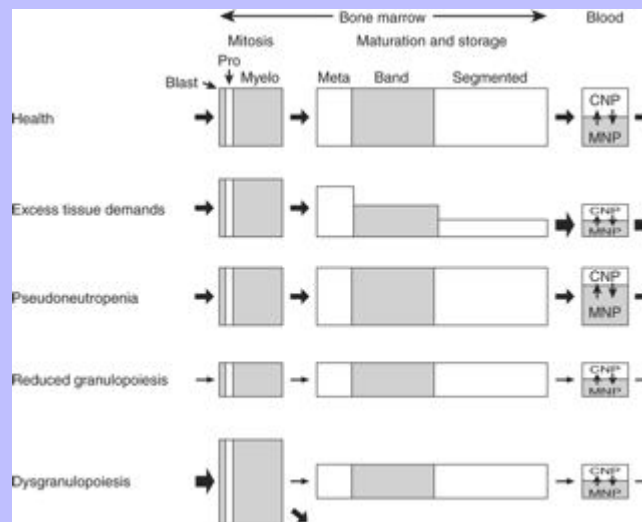
Decreased release of neutrophils from bone marrow can result from decreased numbers of progenitor cells or from abnormal precursor cell maturation called *dysgranulopoiesis*. Conditions in which neutrophil precursors are present in normal or increased numbers in bone marrow but the release of mature neutrophils into blood is decreased include some acute myeloid leukemias, some myelodysplastic syndromes, feline leukemia virus (FeLV) infections, and feline immunodeficiency virus (FIV) infections. Leukopenia has been reported in young dogs with inherited cobalamin deficiency and dysgranulopoiesis in the marrow.

Decreased numbers of neutrophil precursors can occur in bone marrow when generalized marrow hypoplasia or selective neutrophil hypoplasia is present. Hypoplastic conditions associated with decreased numbers of neutrophil precursor cells in the marrow include idiosyncratic drug reactions (e.g., reactions to phenylbutazone, trimethoprim/sulfadiazine, griseofulvin, and cephalosporins), estrogen toxicity (exogenous or endogenous) in dogs and ferrets, cytotoxic chemotherapy drugs, viral diseases (e.g., parvovirus in dogs and cats and equine herpesvirus-1 in foals), *Ehrlichia canis* infection in dogs, and inherited disorders (cyclic hematopoiesis in gray

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collie dogs and Chédiak-Higashi syndrome in some cats). Lithium carbonate stimulates neutrophil production in dogs and humans, but it causes a bone marrow neutrophilic maturation arrest and neutropenia in cats.

Fig. 5-8



Mechanisms responsible for the production of a neutropenia. *CNP*, Circulating neutrophil pool; *MNP*, marginating neutrophil pool.

Decreased numbers of neutrophil precursors can also occur in bone marrow when myelophthisis is present. Myelophthisic disorders are characterized by the replacement of normal hematopoietic cells with abnormal ones. Examples of myelophthisic disorders with decreased numbers of neutrophil precursors in marrow include lymphoid leukemias; multiple myeloma; some myeloid leukemias; some myelodysplastic syndromes; myelofibrosis (often associated with anemia, but less often with leukopenia or thrombocytopenia); osteosclerosis; and metastasis of lymphomas, carcinomas, and mast cell tumors. In myelophthisic disorders, abnormal cells do not simply “crowd out” normal cells; the marrow microenvironment is also altered so that normal hematopoiesis is compromised.

Primary immune-mediated neutropenia is difficult to diagnose in the absence of readily available and reliable diagnostic tests. Animals may be asymptomatic or may be ill because of secondary bacterial infections. Some drug-induced neutropenias and some neutropenias associated with infectious agents probably also have an immune-mediated pathogenetic component. The long-term use of a recombinant G-CSF from one species in a second species can result in a persistent neutropenia in the second species, when antibodies made against the recombinant G-CSF also neutralize the endogenous G-CSF of the species receiving treatment.

Neutropenia can develop in acute inflammatory conditions when the demand for neutrophils depletes the bone marrow storage pool and insufficient time has elapsed for increased granulopoiesis to occur. Neutropenia is common in overwhelming septic conditions (e.g., septicemia) and as a result of endotoxemia. Degenerative left shifts are often present in these disorders. A common example of this type of presentation is acute salmonellosis in horses. Although not well documented in animals, neutropenia may also develop as a result of immune-mediated phagocytosis or hypersplenism. Finally, neutropenia can occur after the net movement of neutrophils from the CNP to the MNP, as occurs during shock.

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## 6.5 INHERITED NEUTROPHIL DEFECTS

### 6.5.1 Chédiak-Higashi Syndrome

Chédiak-Higashi syndrome has been reported in cattle, Persian cats, Aleutian mink, beige mice, blue and silver foxes, and a killer whale, as well as in humans. This disorder is characterized by partial oculocutaneous albinism, increased susceptibility to infections, hemorrhagic tendencies, and the presence of enlarged membrane-bound granules in many cell types, including blood leukocytes. The giant granules may arise from unregulated fusion of primary lysosomes during cell development. Neutrophils from affected animals exhibit reduced mobility and defective phagocytic and/or bactericidal responses, which explains the increased susceptibility to bacterial infections. Neutropenia is a common finding in cats with Chédiak-Higashi syndrome. An increased bleeding tendency is also present because platelets lack normal dense granules, resulting in a platelet storage pool deficiency.

### 6.5.2 $\beta_2$ Integrin Adhesion Molecule Deficiencies

An autosomal recessive deficiency in leukocyte surface adhesion glycoproteins ( $\beta_2$  integrins), resulting from a defect in the CD18  $\beta$  subunit, has been recognized in Irish setters and Holstein cattle. This defect results in decreased neutrophil adhesion, impaired chemotaxis and aggregation, and minimal bactericidal activity. Similar defects also occur in monocytes. As a result, animals have recurrent bacterial and fungal infections without pus formation. Clinical signs include gingivitis, oral ulcers, periodontitis, chronic pneumonia, poor wound healing, and stunted growth. Marked neutrophilia with or without a modest left shift is usually present. Increased numbers of other blood leukocyte types may also occur at times. Mild to moderate nonregenerative anemia and polyclonal hyperglobulinemia may be present.

### 6.5.3 Unknown Neutrophil Function Defects

A less well-defined defect in neutrophils has been reported in Doberman pinschers. Neutrophil chemotaxis and phagocytosis are normal, but these cells have reduced bactericidal ability. The bactericidal defect appears to be the result of inadequate generation of superoxide radicals after stimulation of neutrophils. An inadequate oxidant burst may also occur in young Weimaraner dogs that have recurrent infections.

### 6.5.4 Cyclic Hematopoiesis

Cyclic hematopoiesis (previously termed *cyclic neutropenia*) is transmitted as an autosomal recessive trait in gray collies. The “gray collie syndrome” is associated with several distinct abnormalities (abnormal hair pigmentation, bilateral scleral ectasia, enteropathy, and gonadal hypoplasia) in addition to cyclic hematopoiesis. The specific cause of the disorder is unknown, but it may involve abnormal signal transduction in stem cells in response to a hematopoietic growth factor or factors. Blood neutrophil, monocyte, eosinophil, reticulocyte, and platelet counts exhibit 11- to 13-day cyclic fluctuations, with neutrophil fluctuations being most dramatic. Neutrophils may be completely absent from blood during neutropenic episodes, which last 2 to 4 days. Neutrophilia may follow the neutropenic period. Affected pups are susceptible to bacterial and fungal infections during the neutropenic episodes. They usually die by 6 months of age. Animals that reach adulthood often die of systemic amyloidosis.

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## 6.6 ABNORMAL LYMPHOCYTE MORPHOLOGY

### 6.6.1 Antigenic Stimulation

Lymphocytes proliferate in response to antigenic stimulation. They increase in size, have less dense nuclear chromatin, and exhibit increased cytoplasmic basophilia (Plate 13). Most of these antigenically stimulated cells remain in peripheral lymphoid tissues, but some may enter the circulation, although usually in low numbers. Various terms, including *reactive lymphocytes*, *transformed lymphocytes*, and *immunocytes*, have been used to describe them. Some reactive lymphocytes are large with pleomorphic nuclei and others are plasmacytoid (plasma cell-like) in appearance. Plasma cells are present in lymphoid organs, but they are rarely observed in blood, even when plasma cell neoplasia (e.g., multiple myeloma) is present. Plasma cells have a lower nucleus to cytoplasm ratio and greater cytoplasmic basophilia than resting lymphocytes. The presence of a prominent Golgi complex may create a pale perinuclear area in the cytoplasm. Plasma cells typically have eccentrically located nuclei with coarse chromatin clumping in a mosaic pattern. When it is not possible to determine whether basophilic lymphocytes are reactive or neoplastic, the term *atypical lymphocytes* is sometimes used.

### 6.6.2 Lymphoblasts

Basophilic lymphoid cells are called *lymphoblasts* when a nucleolus or multiple nucleoli are present in the nucleus. Rare lymphoblasts may be observed in disorders with increased antigenic stimulation, but when several of these cells are found during a differential count, lymphoid neoplasia should be suspected.

### 6.6.3 Cytoplasmic Granules and Vacuoles

A low percentage of lymphocytes in blood from healthy animals contains cytoplasmic granules (Plate 17). These granular lymphocytes may be cytotoxic T lymphocytes or NK cells. Increased numbers of granular lymphocytes have been seen in both neoplastic and nonneoplastic conditions. Basophilic granules may be seen in the lymphocytes from animals with certain lysosomal storage diseases, including mucopolysaccharidosis VI and VII in dogs and cats and GM<sub>2</sub> gangliosidosis in pigs. Lymphocytes, as well as other blood cell types, may also contain distemper inclusions.

Cytoplasmic vacuoles may be seen in lymphocytes obtained from animals with a variety of neoplastic and nonneoplastic disorders. Discrete vacuoles may be observed in the cytoplasm of lymphocytes from animals with inherited lysosomal storage diseases, including cats with mucopolysaccharidosis VII, cats with GM<sub>2</sub> gangliosidosis, cats and dogs with GM<sub>1</sub> gangliosidosis, cats with  $\alpha$ -mannosidosis, goats with  $\beta$ -mannosidosis, cats with Niemann-Pick disease type C, and dogs with  $\alpha$ -l-fucosidase deficiency. Basophilic granules and vacuoles may not become apparent in some lysosomal disorders until the affected animal reaches adulthood.

## 6.7 LYMPHOCYTOSIS

Lymphocyte numbers in blood vary with age. Absolute lymphocyte counts are higher in adults than in young animals of some species (e.g., horses) and lower in adults than in young animals of other species (e.g., cats). Lymphocytosis sometimes occurs with epinephrine release in animals (especially horses and cats), presumably because of transiently increased lymphatic flow associated with increased muscular contractions. Although increased proliferation of lymphocytes is common in lymph nodes during response to foreign antigens, evidence of

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this reaction is often not present in blood. In some cases, reactive lymphocytes account for a substantial proportion of the total lymphocytes present in blood, but absolute lymphocytosis is uncommon. Vaccine reaction in young dogs is an instance in which reactive lymphocytosis may be observed. Lymphocytosis is sometimes present in animals with chronic inflammatory conditions.

Persistent lymphocytosis often occurs in cattle infected with the bovine leukemia virus, a B-lymphotropic retrovirus. The lymphocytosis is due to spontaneous proliferation of B lymphocytes, which reportedly do not express virus. Instead, it is postulated that they are stimulated to proliferate by a second B-lymphocyte population that does express virus or activated helper (CD4<sup>+</sup>) T lymphocytes. Persistent lymphocytosis is a subclinical condition in cattle, but lymphoma will subsequently develop in some animals.

Marked lymphocytosis is always present in animals with chronic lymphocytic leukemia (CLL). Lymphocytosis often occurs in animals with acute lymphoblastic leukemia and occasionally occurs in animals with lymphoma (see lymphoproliferative disorders section). Granular lymphocytes may be increased in response to infectious agents (e.g., *Ehrlichia canis*) or in association with neoplastic disorders involving these cells.

## 6.8 LYMPHOPENIA

Lymphocytes are sequestered in bone marrow, lymph nodes, and the spleen after endogenous release of or exogenous administration of glucocorticoid steroids. Glucocorticoids can also potentiate apoptosis of sensitive lymphocytes (e.g., transformed lymphocytes or neoplastic lymphocytes). The release of endogenous glucocorticoids in response to severe systemic disorders may play a large role in the production of lymphopenia that often accompanies these disorders. Lymphopenia occurs after experimental injection of IL-1 and TNF, and lymphopenia often accompanies severe systemic bacterial infections. Lymphopenia and lymphadenopathy are also induced by interferons released in response to viral infections and immune stimulation. The lymphopenia that develops in response to interferons apparently results from decreased egress of lymphocytes from lymphoid tissues into the efferent lymph. Lymphopenia often occurs after the use of immunosuppressive drugs and irradiation that result in lymphocyte destruction.

Lymphocytes are present in afferent lymph from gastrointestinal and bronchial lymphoid tissues and efferent lymph from lymph nodes. The loss of lymphocyte-rich afferent lymph (e.g., lymphangiectasia) or efferent lymph (e.g., thoracic duct rupture) results in lymphopenia because most blood lymphocytes recirculate through lymphoid tissues. Lymphopenia can also occur when lymph node architecture is disrupted (e.g., multicentric lymphoma or generalized granulomatous inflammation), preventing the normal recirculation of lymphocytes.

Lymphopenia occurs with hereditary T-lymphocyte deficiency or combined T- and B-lymphocyte deficiency, because T lymphocytes account for the majority of lymphocytes normally present in the circulation. Profound lymphopenia occurs in Arabian foals with severe combined immunodeficiency that affects both the T- and B-lymphocyte lineages.

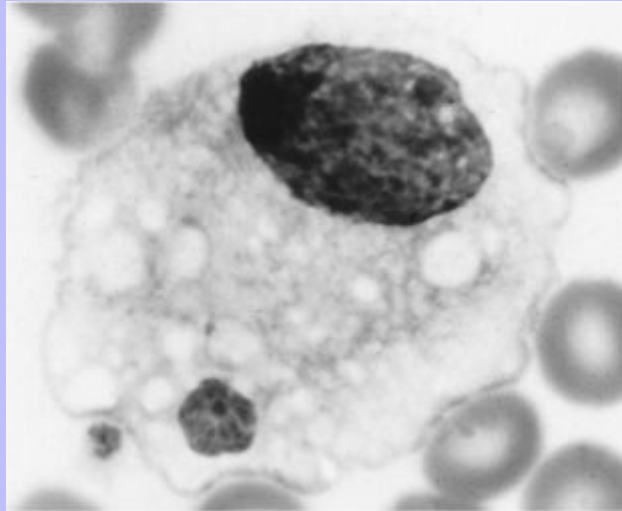
## 6.9 ABNORMAL MONOCYTE MORPHOLOGY

Cytoplasmic vacuoles have been reported in some animals with lysosomal storage disorders, but monocytes from healthy individuals often exhibit cytoplasmic vacuoles on films prepared from blood collected with an anticoagulant. Transformation of monocytes into macrophages typically occurs in the tissues, but macrophages are sometimes seen in blood (Fig. 5-9). In addition to other blood cell types, canine monocytes may contain distemper inclusions, and monocytes from animals with Chédiak-Higashi syndrome may have one or more enlarged eosinophilic granules in the cytoplasm. Morulae of *Anaplasmatidae* organisms that infect mononuclear

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phagocytes (e.g., *Ehrlichia canis* and *Neorickettsia risticii*) can rarely be found in blood monocytes or macrophages during the acute stage of infection (see Fig. 5-9). These morulae appear as tightly packed basophilic clusters of organisms within the cytoplasm. Other infectious agents that are rarely seen in blood mononuclear phagocytes include *Histoplasma capsulatum*, *Mycobacterium* species, *Leishmania infantum*, and remarkably large schizonts of *Cytosuxoon felis*.

Fig. 5-9



Dog blood macrophage containing an *Ehrlichia canis* morula. (Wright-Giemsa stain.)

### 6.10 MONOCYTOSIS

Monocytosis may occur in conditions that also cause neutrophilia. It may be present in both acute and chronic inflammation. Glucocorticoid steroids can induce monocytosis in dogs. Healthy domestic animals may have few or no monocytes in blood; consequently, the term *monocytopenia* is not usually used. Monocytosis may occur in animals with monocytic or myelomonocytic leukemias. In contrast to other causes of monocytosis, high numbers of monoblasts will be present in bone marrow (and often in blood) of animals with these leukemias.

### 6.11 EOSINOPHILIA

Eosinophilia occurs in disorders that result in increased IL-5 production. Eosinophilia may accompany parasitic diseases, especially those caused by nematodes and flukes. Eosinophilia is more likely present when intestinal nematodes are migrating within the body than when they are only located within the intestine. Eosinophilia may occur in association with inflammatory conditions of organs that normally contain numerous mast cells, such as skin, lungs, intestine, and uterus. It may be present in animals with IgE-mediated allergic hypersensitivity reactions, such as flea-bite allergies and feline asthma. Although not usually present, eosinophilia may occur in animals with mast cell tumors, but it is rare in animals with other tumor types. Eosinophilia occurs in some animals with eosinophilic granulomas, and it is consistently present in animals with hypereosinophilic syndrome, a heterogeneous group of disorders that can be difficult to differentiate from eosinophilic leukemia. Finally, eosinophilia may accompany CML.

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## 6.12 EOSINOPENIA

The absolute eosinophil count may be zero in some healthy animals, making eosinopenia of limited significance. Glucocorticoids rapidly produce eosinopenia as a result of the sequestration of eosinophils within the bone marrow. Long-term glucocorticoid therapy may decrease eosinophil production by decreasing the production of growth factors from T lymphocytes. Glucocorticoids also potentiate apoptosis of eosinophils in tissues.

## 6.13 BASOPHILIA

Basophilia is generally associated with IgE-mediated disorders. When present, basophilia usually accompanies eosinophilia. Basophilia may occur in some animals with mast cell tumors (primarily noncutaneous types). Basophilia has rarely been reported in association with basophilic leukemia in animals. Basophilic leukemia must be differentiated from mast cell neoplasia with mastocytosis (sometimes called *mast cell leukemia*). Mast cells have round nuclei and basophils have segmented nuclei.

## 6.14 MASTOCYTOSIS

Mast cells are not normally found in blood. They develop in tissues from precursor cells produced in the bone marrow. Mast cells and basophils have similar biochemical characteristics and probably share a common progenitor cell in bone marrow, but they are clearly different cell types. Basophils have segmented nuclei and mast cells have round nuclei. Mastocytosis occurs in association with noncutaneous and metastatic cutaneous mast cell tumors. Mast cells may also be present in the blood of animals with inflammatory diseases, regenerative anemia, tissue injury, neoplasia other than mast cell tumors, and necrosis.

## 6.15 HEMATOPOIETIC NEOPLASMS

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Hematopoietic neoplasms arise from bone marrow, lymph nodes, the spleen, or the thymus. They are classified as either lymphoproliferative disorders or myeloproliferative disorders. The term *leukemia* is used when neoplastic cells are seen in blood, bone marrow, or both. An exception is the neoplastic proliferation of plasma cells in bone marrow (multiple myeloma), which is not referred to as a leukemia. True leukemias are of bone marrow origin and neoplastic cells are usually present in blood. Leukocyte counts may be low, normal, or high in animals with leukemias. The term *acute* is used to describe leukemias in which a predominance of blast cells occurs in the bone marrow, and the term *chronic* is used for leukemias in which there is a predominance of well-differentiated cells in blood and bone marrow. The progression of disease is usually rapid (weeks to months) in acute leukemias and slow (months to years) in chronic leukemias.

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### 6.15.1 Lymphoproliferative Disorders

The term *lymphoma* denotes the presence of a solid tumor or tumors composed of neoplastic lymphocytes located outside the bone marrow. The term *lymphoid leukemia* indicates a neoplastic condition of lymphocytes present in bone marrow, blood, or both that is not associated with a solid tumor or tumors. Lymphoid leukemias are further classified as acute or chronic, depending on the maturity of the cells involved. When neoplastic cells are present in the blood of an animal with a lymphoma, the terms *lymphoma with leukemia* or *lymphosarcoma cell leukemia* are used, but the former term is preferred. Metastasis from bone marrow to lymphoid tissues or from lymphoid tissues to bone marrow is common. Consequently, it may be difficult to differentiate a true leukemia from a lymphoma with leukemia in animals with advanced stages of disease.

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## 6.15.1.1 Acute Lymphoblastic Leukemia

Neoplastic lymphoblasts and/or prolymphocytes are present in bone marrow of animals with acute lymphoblastic leukemia (ALL). Neoplastic cells are also usually present in blood, with or without an absolute lymphocytosis. Pancytopenia has been recognized in horses and dogs with ALL diagnosed by bone marrow biopsy. Lymphoblasts are generally difficult to differentiate from blast cells of other hematopoietic lineages without the use of special stains or surface markers. In cats with ALL, the neoplastic cells usually have the T-lymphocyte phenotype. Most cats with ALL are also FeLV-positive; however, ALL has been reported in FIV-positive, FeLV-negative cats. The neoplastic cells of dogs with ALL may be of the T-lymphocyte, B-lymphocyte, NK cell, or null cell phenotypes. In humans, the terms *precursor B lymphoblastic leukemia* and *precursor T lymphoblastic leukemia* have been proposed for B-lymphocyte type ALL and T-lymphocyte type ALL, respectively.

## 6.15.1.2 Chronic Lymphocytic Leukemia

CLL is reported most often in old animals. Lymphocytosis, involving normal-appearing lymphocytes, is consistently present in blood. In contrast to cats with ALL, most cats with CLL are FeLV-negative.

T-lymphocyte, B-lymphocyte, and large granular lymphocyte (LGL) types of CLL have been identified. Dogs with B-lymphocyte type CLL often have an accompanying monoclonal gammopathy, which is most often of the IgM type. An IgG monoclonal gammopathy has also been reported in a horse with CLL.

Lymphocytes present in the LGL type of CLL have red- or purple-staining (generally focal) granules within light-blue cytoplasm. Although disease progression in most dogs with LGL leukemia is similar to that of CLL, progressing slowly over several years, the disease in some dogs with LGL leukemia behaves like an aggressive form of ALL, progressing rapidly over weeks to a few months. Nearly all dogs with the LGL form of CLL have neoplastic T lymphocytes, but the neoplastic cells of dogs with the LGL form of ALL may be of either the T-lymphocyte or the NK cell type.

Bone marrow examination of animals with CLL often reveals increased numbers of normal-appearing lymphocytes; however, the extent of infiltration is generally less than that seen in ALL. Although B-lymphocyte type CLL may originate in the bone marrow, T-lymphocyte CLL appears to develop outside the marrow (e.g., in the spleen) with secondary marrow infiltration. Special surface marker tests are usually needed to identify the cell type involved. However, the coexistence of a monoclonal gammopathy with CLL indicates that the neoplasia is of the B-lymphocyte type.

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## 6.15.1.3 Lymphomas

Lymphomas are solid tumors of neoplastic lymphocytes that develop outside the bone marrow. They may be classified by the anatomic site involved (e.g., alimentary, thymic, cutaneous, multicentric), by cell location in lymph nodes (e.g., follicular, mantle cell, diffuse), by stage of development (e.g., precursor, prolymphocytic, small lymphocytic), and by cell type (e.g., B-lymphocyte, T-lymphocyte, NK cell). A World Health Organization (WHO) classification of tumors group has proposed criteria for classification of lymphomas that will require evaluation by the veterinary community to determine how applicable the various classifications are for animal lymphomas in various species. Neoplastic cells are recognized by their abnormal morphology in the blood of about one quarter to one half of the animals presenting with a

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lymphoma. Bone marrow infiltrates may sometimes be recognized in animals even when neoplastic cells are not appreciated in blood.

Lymphomas involving LGLs have been primarily reported in cats, in which they generally occur as intestinal lymphomas that metastasize to various other organs, including the spleen, liver, lymph nodes, blood, and bone marrow. Their granules are generally much larger than those seen in normal LGLs in blood. The neoplastic cells may originate from intraepithelial lymphocytes, and most, but not all, of these tumors appear to be composed of cytotoxic T lymphocytes.

### 6.15.1.4 Multiple Myeloma

Multiple myeloma (plasma cell myeloma) is a B-lymphocyte tumor of bone marrow that is manifested as a proliferation of plasma cells. A monoclonal IgG or IgA immunoglobulin is usually secreted by the tumor, resulting in a monoclonal hyperglobulinemia, which is detected by means of serum protein electrophoresis (see [Chapter 9](#)). The class of immunoglobulin produced (e.g., IgG) can be identified by using immunoelectrophoresis and quantified by using methods such as single radial immunodiffusion. Rarely, multiple myeloma secretes no measurable monoclonal protein, produces biclonal proteins, or produces only a component of an immunoglobulin molecule (light chains or heavy chains). Focal lytic or diffuse osteoporotic bone lesions are often detected by means of survey radiography, and a Bence-Jones proteinuria (immunoglobulin light chains in urine) may be present. Plasma cell infiltrates may be found in other tissues including the spleen, liver, lymph nodes, and kidneys.

### 6.15.1.5 Plasmacytoma

In addition to the metastasis of multiple myeloma from bone marrow, extramedullary plasma cell tumors (plasmacytomas) may arise as primary tumors of soft tissues. They occur most frequently as benign solitary tumors in the gastrointestinal tract, oral cavity, skin, digits of the forelegs, and ears of dogs. Plasmacytomas rarely have an associated monoclonal or biclonal hyperglobulinemia and rarely metastasize to distant sites in dogs. Plasmacytomas appear more aggressive and likely to metastasize in cats, and it may be difficult to differentiate metastatic plasmacytoma from metastatic multiple myeloma in cats.

### 6.15.1.6 Other Immunoproliferative B-Lymphocyte Neoplasms

Any cell type in the normal B-lymphocyte maturation pathway may become neoplastic and produce an immunoglobulin. The nature of an immunoproliferative disorder is determined by the stage at which B-lymphocyte maturation is arrested. Other B-lymphocyte neoplasms, including multicentric lymphomas, B-lymphocyte CLL (discussed previously), and primary macroglobulinemia may produce monoclonal hyperglobulinemias. Lytic bone lesions are generally absent in these disorders, even when bone marrow infiltrates are present. Primary (Waldenström's) macroglobulinemia is characterized as a lymphoplasmacytic neoplasm that produces an IgM monoclonal protein. This syndrome is rarely reported in animals. The spleen, liver, and lymphoid tissues, rather than bone marrow, may have neoplastic infiltrates in some cases.

### 6.15.2 Myeloproliferative Disorders

Myeloproliferative disorders (MPDs) are neoplastic diseases characterized by the purposeless proliferation of one or more of the nonlymphoid marrow cell lines (granulocytic, monocytic, erythrocytic, or megakaryocytic). Primary myelodysplastic syndromes (MDSs) are also considered to be MPDs because primary MDSs have been

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shown to be clonal and may precede the development of acute myeloid leukemia (AML). The unitary concept of MPD was developed, because all nonlymphoid blood cells are derived from a common myeloid stem cell and neoplastic transformations in these disorders usually occur in pluripotent progenitor cells. Although the proliferation of one cell type may predominate, a marrow cell line is seldom singly affected. Morphologic or functional disorders of other nonlymphoid cell lines can usually be detected. In addition, some of these disorders appear to evolve into one another. For example, in cats with MDS with excessive proliferation of nucleated erythrocytes (MDS-Er), the disease may evolve into erythroleukemia (AML-M6) and eventually into AML-M1 or AML-M2.

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MPDs are classified by the percentages of various blast cell types in the bone marrow and numbers of differentiated cells in the peripheral blood. The term *acute* is used to describe MPDs in which nonlymphoid blast cells account for more than 30% of all nucleated cells in the marrow. The term *chronic* is used for MPDs in which nonlymphoid blast cells account for less than 30% of all nucleated cells in the marrow and high numbers of differentiated nonlymphoid cells are found in blood, as well as bone marrow. A WHO classification of tumors group has recently recommended that the cutoff value for acute leukemia be reduced from 30% to 20% blast cells in humans. A similar reduction is under consideration for animals at the time of this writing. The progression of disease is usually rapid (weeks to months) in acute MPDs and slow (months to years) in chronic MPDs. Cats with MPD are generally infected with FeLV, FIV, or both. MPDs are uncommon in other domestic animal species and the causes are unknown. In experiments, irradiation has been shown to cause MPD in dogs.

6.15.2.1

## Hematologic Findings

Excluding primary erythrocytosis and thrombocythemia, abnormalities that may be present in blood include nonregenerative anemia with erythrocyte macrocytosis, anisocytosis, or poikilocytosis; nucleated erythrocytes out of proportion to the number of reticulocytes present and/or nucleated erythrocytes with lobulated or fragmented nuclei; large bizarre or hypogranular platelets; and immature granulocytes or granulocytes with abnormal structure (large size, hyposegmentation, hypersegmentation). Nonlymphoid blast cells may be present in blood, depending on the type of MPD and stage of development. The platelet count is frequently low but may be normal or high. The total leukocyte counts and absolute neutrophil, monocyte, eosinophil, and basophil counts vary from low to high, depending on the MPD that is present. Although a preliminary diagnosis of MPD can sometimes be made on the basis of hematologic findings, a definitive diagnosis and classification requires bone marrow evaluation.

6.15.2.2

## Myelodysplastic Syndromes

The bone marrow in MDS is usually normocellular or hypercellular, but cytopenias (especially anemia and thrombocytopenia) are present in blood. This apparent ineffective hematopoiesis appears to result from extensive apoptosis. Apoptosis, or programmed cell death, is a physiologic mechanism of gene-directed cellular self-destruction in which intracellular endonucleases initially cut DNA into fragments. Recognizable apoptotic cells with fragmented nuclei exist for only 10 to 15 minutes before they are removed by phagocytic cells.

Evidence of dyserythropoiesis, dysgranulopoiesis, and/or dysmegakaryocytopoiesis is present in the marrow. Erythroid abnormalities that may be present include megaloblastic cells, abnormal nuclear shapes, premature nuclear pyknosis, nuclear fragmentation, multinucleated cells, nuclear and cytoplasmic asynchrony, maturation arrest, and siderotic inclusions. Neutrophilic abnormalities that may be present include increased numbers (5% to 29%) of myeloblasts; maturation arrest in the neutrophilic series at the

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myelocyte-metamyelocyte stage; giant metamyelocytes, bands, and mature neutrophils; abnormal granulation such as large primary granules or granules surrounded by vacuoles; hyposegmented neutrophils (pseudo-Pelger-Huët); hypersegmented neutrophils; and neutrophils with bizarre nuclear shapes. Increased numbers of eosinophilic cells are also commonly observed in MDSs in cats. Megakaryocytic abnormalities that may be present include dwarf granular megakaryocytes with single or multiple nuclei and large megakaryocytes with nuclear abnormalities including hypolobulation, hyperlobulation, and multiple round nuclei. Some cats with MDSs have stainable iron in their marrow. In contrast to bone marrow in dogs, normal cat bone marrow does not exhibit stainable iron.

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Classification of MDSs into three subtypes according to blood and bone marrow findings has been suggested in animals. MDSs with erythroid predominance in the bone marrow (myeloid/erythroid [M/E] ratio below 1) may be classified as MDS-Er. Cases previously diagnosed as erythremic myelosis would now be placed in this category, as long as the number of blast cells in the marrow was less than 30% of all nucleated cells. Cases with refractory anemia and M/E ratio above 1, with or without other refractory cytopenias, may be described as myelodysplastic syndrome–refractory cytopenia (MDS-RC). Myeloblasts account for less than 5% of all nucleated cells in this subtype. When myeloblasts are increased (5% to 29% of bone marrow nucleated cells), the term *myelodysplastic syndrome-excess blast (MDS-EB)* may be used. Animals with bone marrow blast counts slightly below 30% may represent animals in transition between MDS and AML, or they may represent animals that have AML that is not diagnosed as such, because the blast count does not exceed the arbitrary number previously established. A WHO classification of tumors group has proposed various subtypes of MDS. Once these proposals are reviewed by veterinary clinical pathologists and veterinary oncologists and compared with animal MDS presentations, a new universally accepted classification scheme may be adopted in veterinary medicine.

6.15.2.3

## Acute Myeloid Leukemias

A classification system for AML in dogs and cats has been proposed by the American Society for Veterinary Clinical Pathology Animal Leukemia Study Group. AML is diagnosed when the percentage of nonlymphoid hematopoietic blast cells in the bone marrow equals or exceeds 30% of all nucleated cells, excluding lymphocytes, macrophages, plasma cells, and mast cells. Dyserythropoiesis, dysgranulopoiesis, and/or dysmegakaryocytopoiesis are also frequently present, with megaloblastic nucleated erythroid cells being most commonly observed. If blast cells account for less than 30% of all nucleated cells and dysplastic changes are present, a diagnosis of MDS is made. As discussed previously, this cutoff percentage for blast cells may be reduced from 30% to 20% in the future for animals, as it has been for humans. Cytochemistry, immunocytochemistry, and electron microscopy may be used to help identify the type(s) of blast cells present to classify the type of AML present (Fig. 5-10). In addition to being quantified as a percentage of all nucleated cells, cells may also be quantified on the basis of the total number of nonerythroid cells (NECs), which is determined by subtracting the nucleated erythroid cells from the count of all nucleated cells.

Myeloblastic leukemia without maturation is designated *AML-M1*, and myeloblastic leukemia with differentiation is designated *AML-M2*. Variants of *AML-M2* with basophilic differentiation and eosinophilic differentiation have been classified as *AML-M2-B* and *AML-M2-Eos*, respectively. Acute myelomonocytic leukemia is designated *AML-M4*, and acute monocytic leukemia is designated *AML-M5*. Erythroleukemia is designated *AML-M6*. In contrast to the subtypes of AML discussed previously, in *AML-M6* the M/E ratio is less than 1.0. The designation *AML-M6Er* is used when the M/E ratio is less than 1.0 and rubriblasts are included in the blast count to equal or exceed 30% of all nucleated cells. Megakaryoblastic leukemia is designated *AML-M7*. Finally, acute undifferentiated leukemia (AUL) is diagnosed when the blast cells cannot be identified with certainty by using routine blood stains or cytochemical markers. This term may be

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used as a temporary category in some cases, pending the use of specialized cell markers. The hallmark of AUL is the presence of blast cells with broad cytoplasmic pseudopods and/or some magenta-staining cytoplasmic granules. Cats with an MPD previously referred to as *reticuloendotheliosis* may be included in this category or in AML-M6Er.

6.15.2.4

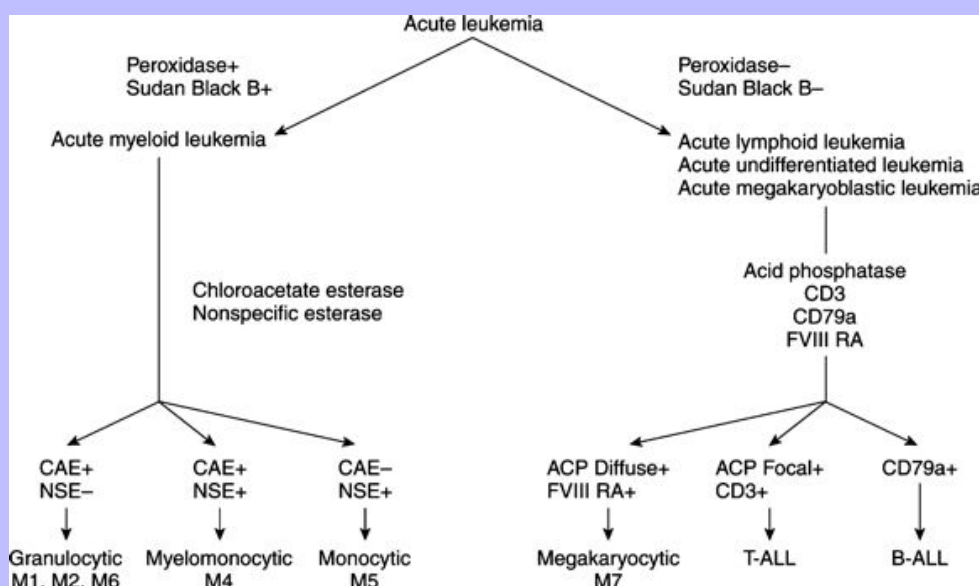
### Chronic Myeloproliferative Disorders

Chronic MPDs are neoplastic proliferations of hematopoietic cells resulting in high numbers of differentiated cells in blood. Like animals with MDS, animals with chronic MPD have less than 30% blast cells in the bone marrow and few or no blast cells in the blood. Dysplastic changes may be present in both disorders, but they tend to be more pronounced in MDS. The major difference between these disorders is that high numbers of one or more blood cell types occur in chronic MPD and cytopenias frequently occur in MDS.

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Fig. 5-10



Simplified classification of acute leukemia with cytochemical stains and immunocytochemistry. *FVIII RA*, Factor VIII-related antigen; *T-ALL*, T-cell acute lymphoblastic leukemia; *B-ALL*, B-cell acute lymphoblastic leukemia.

CML is a rare disorder in dogs, and possible cases in cats have not been well documented. CML presents with a high total leukocyte count (greater than 50,000/ $\mu$ L) with a marked neutrophilic left shift in blood. Increased numbers of monocytes, eosinophils, and/or basophils may also be present. If monocytes predominate, a diagnosis of chronic monocytic leukemia may be considered. Myeloblasts are either absent or present in low numbers in blood. CML must be differentiated from severe inflammatory leukemoid reactions. Chronic myelomonocytic leukemia has been considered a variant of CML in humans but is now generally classified as a form of MDS. Eosinophilic leukemia is a variant of CML in which eosinophilic cells predominate in blood and marrow. Differentiation of eosinophilic leukemia and the hypereosinophilic syndrome in cats can be difficult. Basophilic leukemia is a variant of CML in which basophilic cells

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predominate in blood and marrow. It has been reported in dogs, but not in other domestic animals. Early reports of basophilic leukemia in animals represented misdiagnosed cases of systemic mastocytosis with mastocythemia.

Primary erythrocytosis (polycythemia vera) is considered to be a chronic MPD that results from an autonomous (erythropoietin-independent) proliferation of erythroid precursor cells, resulting in high numbers of mature erythrocytes in blood. In contrast to humans, domestic animals with polycythemia vera do not generally have increased granulocyte and platelet numbers. The bone marrow is hyperplastic with orderly maturation of cells. The M/E ratio is often normal but may be decreased as a result of erythroid hyperplasia. A diagnosis of primary erythrocytosis is ultimately made by ruling out causes of secondary erythrocytosis.

Thrombocythemia is an MPD that is characterized by persistent, markedly increased (usually above  $1 \times 10^6/\mu\text{L}$ ) platelet counts and megakaryocytic hyperplasia in the bone marrow. Megakaryocyte morphology may appear normal or dysplastic, when examined by means of light microscopy. A diagnosis of thrombocythemia is ultimately made by ruling out other causes of the high platelet count.

## 6.16 REFERENCES

JC Bloom, PA Thiem, TS Sellers, et al.: Cephalosporin-induced immune cytopenia in the dog: Demonstration of erythrocyte-, neutrophil-, and platelet-associated IgG following treatment with cefazidone [published erratum appears in *Am J Hematol* Dec 1988;29(4):241]. *Am J Hematol.* **28**, 1988, 71–78.

JD Brain, RM Molina, MM DeCamp, et al.: Pulmonary intravascular macrophages: Their contribution to the mononuclear phagocyte system in 13 species. *Am J Physiol.* **276**, 1999, L146–L154.

MR Brown, KS Rogers: Neutropenia in dogs and cats: A retrospective study of 261 cases. *J Am Anim Hosp Assoc.* **37**, 2001, 131–139.

CG Couto: Immune-mediated neutropenia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 815–818.

RL Cowell, LS Decker: Interpretation of feline leukocyte responses. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 382–390.

JC Fyfe: Hematology of selective intestinal cobalamin malabsorption. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 965–970.

SD Gaunt: Extreme neutrophilic leukocytosis. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 347–355.

JM Gayle, ND Cohen, MK Chaffin: Factors associated with survival in septicemic foals: 65 cases (1988–1995). *J Vet Intern Med.* **12**, 1998, 140–146.

U Giger: Hereditary blood diseases. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 955–959.

CL Greenfield, JB Messick, PF Solter, et al.: Results of hematologic analyses and prevalence of physiologic leukopenia in Belgian Tervuren. *J Am Vet Med Assoc.* **216**, 2000, 866–871.

JW Harvey: Atlas of veterinary hematology. In *Blood and bone marrow of domestic animals*. 2001, WB Saunders Co., Philadelphia.

104

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# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- KL Henson, RE Raskin: Myeloproliferative disorders. In Morgan, RV, Bright, RM, Swartout, MS (Eds.): *Handbook of small animal practice*. 4th ed., 2003, Saunders/Elsevier Science, Philadelphia, 669–676.
- U Hinrichs, S Puhl, GR Rutteman, et al.: Lymphangiosarcomas in cats: A retrospective study of 12 cases. *Vet Pathol*. **36**, 1999, 164–167.
- BH Horwitz, JP Mizgerd, ML Scott, et al.: Mechanisms of granulocytosis in the absence of CD18. *Blood*. **97**, 2001, 1578–1583.
- ES Jaffe, NL Harris, H Stein, et al.: In *Pathology and genetics of tumors of haematopoietic and lymphoid tissues*. 2001, IARC Press, Lyon, France.
- NC Jain, JT Blue, CB Grindem, et al.: Proposed criteria for classification of acute myeloid leukemia in dogs and cats. *Vet Clin Pathol*. **20**, 1991, 63–82.
- CM Knottenbelt, JW Simpson, ML Chandler: Neutrophilic leucocytosis in a dog with a rectal tumour. *J Small Anim Pract*. **41**, 2000, 457–460.
- KS Latimer: Pelger-Huët anomaly. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 976–983.
- KS Latimer, RP Campagnoli, DM Danilenko: Pelger-Huët anomaly in Australian shepherds: 87 cases (1991-1997). *Comp Haematol Int*. **10**, 2000, 9–13.
- M Lorenz, WE Evering, A Provencher, et al.: Atypical antipsychotic-induced neutropenia in dogs. *Toxicol Appl Pharmacol*. **155**, 1999, 227–236.
- MD Lucroy, BR Madewell: Clinical outcome and associated diseases in dogs with leukocytosis and neutrophilia: 118 cases (1996-1998). *J Am Vet Med Assoc*. **214**, 1999, 805–807.
- MD Lucroy, BR Madewall: Clinical outcome and diseases associated with extreme neutrophilic leukocytosis in cats: 104 cases (1991-1999). *J Am Vet Med Assoc*. **218**, 2001, 736–739.
- SP McDonough, PF Moore: Clinical, hematologic, and immunophenotypic characterization of canine large granular lymphocytosis. *Vet Pathol*. **37**, 2000, 637–646.
- PM McManus: Frequency and severity of mastocytemia in dogs with and without mast cell tumors: 120 cases (1995-1997). *J Am Vet Med Assoc*. **215**, 1999, 355–357.
- KM Meyers: Chédiak-Higashi syndrome. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 971–975.
- FM Moore, HS Bender: Neutropenia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 350–355.
- GP Niemeyer, CD Lothrop, Jr.: Cyclic hematopoiesis. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 960–964.
- K Oguma, R Kano, A Hasegawa: In vitro study of neutrophil apoptosis in dogs. *Vet Immunol Immunopathol*. **76**, 2000, 157–162.
- D Peeters, C Clercx, A Thiry, et al.: Resolution of paraneoplastic leukocytosis and hypertrophic osteopathy after resection of a renal transitional cell carcinoma producing granulocyte-macrophage colony-stimulating factor in a young bull terrier. *J Vet Intern Med*. **15**, 2001, 407–411.
- RE Raskin, A Valenciano: Cytochemical tests for diagnosis of leukemia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 755–763.
- JB Rottman: Key role of chemokines and chemokine receptors in inflammation, immunity, neoplasia, and infectious disease. *Vet Pathol*. **36**, 1999, 357–367.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

A Salama, B Schütz, V Kiefel, et al.: Immune-mediated agranulocytosis related to drugs and their metabolites: Mode of sensitization and heterogeneity of antibodies. <i>Br J Haematol.</i> <b>72</b> , 1989, 127–132.	
AE Schultze: Interpretation of canine leukograms. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): <i>Schalm's veterinary hematology</i> . 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 366–381.	
MA Scott, SL Stockham: Basophils and mast cells. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): <i>Schalm's veterinary hematology</i> . 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 308–317.	
LC Sharkey, IJ Rosol, A Gröne, et al.: Production of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor by carcinomas in a dog and a cat with paraneoplastic leukocytosis. <i>J Vet Intern Med.</i> <b>10</b> , 1996, 405–408.	
WP Sheridan, P Hunt, S Simonet, et al.: Hematologic effects of cytokines. In Remick, DG, Friedland, JS (Eds.): <i>Cytokines in health and disease</i> . 2nd ed., 1997, Marcel Dekker, Inc., New York, 487–505.	105
J Shi, H Fujieda, Y Kokubo, et al.: Apoptosis of neutrophils and their elimination by Kupffer cells in rat liver. <i>Hepatology</i> . <b>24</b> , 1996, 1256–1263.	106
JE Smolen, LA Boxer: Functions of neutrophils. In Beutler, E, Lichtman, MA, Coller, BS, et al. (Eds.): <i>Williams hematology</i> . 6th ed., 2001, McGraw-Hill, New York, 761–784.	
DM Stone, LK Norton, JC Chambers, et al.: CD4 T lymphocyte activation in BLV-induced persistent B lymphocytosis in cattle. <i>Clin Immunol.</i> <b>96</b> , 2000, 280–288.	
JA Taylor: Leukocyte responses in ruminants. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): <i>Schalm's veterinary hematology</i> . 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 391–404.	
IR Tizard: In <i>Veterinary immunology. An introduction</i> . 6th ed., 2000, WB Saunders Co., Philadelphia.	
G Trowald-Wigh, S Ekman, K Hansson, et al.: Clinical, radiological and pathological features of 12 Irish setters with canine leucocyte adhesion deficiency. <i>J Small Anim Pract.</i> <b>41</b> , 2000, 211–217.	
AJ Wardlaw, AB Kay: Eosinophils and their disorders. In Beutler, E, Lichtman, MA, Coller, BS, et al. (Eds.): <i>Williams hematology</i> . 6th ed., 2001, McGraw-Hill, New York, 785–799.	
EG Welles: Clinical interpretation of equine leukograms. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): <i>Schalm's veterinary hematology</i> . 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 405–410.	
V Witko-Sarsat, P Rieu, B Descamps-Latscha, et al.: Neutrophils: molecules, functions, and pathophysiological aspects. <i>Lab Invest.</i> <b>80</b> , 2000, 617–653.	
Young, KM: Eosinophils. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): <i>Schalm's veterinary hematology</i> . 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 297–307.	

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## 7 Chapter 6 Evaluation of Hemostasis: Coagulation and Platelet Disorders

Hemostasis depends on vascular integrity, platelet numbers and function, and coagulation. Vascular integrity is determined in large measure by the health of endothelial cells and their extracellular matrix. Damage to vessel walls can result in hemorrhage and/or the activation of platelets and coagulation. When arteries are severed, a transient reflex vasoconstriction slows the loss of blood and allows some time for the commencement of platelet plug formation and coagulation, which eventually result in the formation of a stable thrombus.

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### 7.1 BLOOD PLATELETS

Blood platelets (thrombocytes) in mammals are small, round to oval, anucleated cell fragments that form from cylinders of megakaryocyte cytoplasm. Platelet cytoplasm appears light blue with many small reddish purple granules when it is visualized on routine blood stains. Unstimulated platelets appear as thin disks on examination with scanning electron microscopy. They have a life span of 5 to 10 days in most domestic animals. Normal platelet counts vary depending on the species, with minimal reference values as low as  $100 \times 10^3/\mu\text{L}$  in horses and maximal reference values as high as  $800 \times 10^3/\mu\text{L}$  in several domestic animal species. The numbers normally present in blood greatly exceed those needed for adequate hemostasis. The spleen stores about 30% of the total platelet mass in humans, but splenic contraction after  $\alpha$ -adrenergic stimulation (as occurs during exercise) does not result in a thrombocytosis in horses or dogs.

Thrombocytes in nonmammalian species have nuclei and are much larger than those in mammals. They have a high nuclear to cytoplasmic ratio and are often oval or elongated with light blue cytoplasm on stained blood films. Cytoplasmic vacuoles may be present at one or both ends of a cell. Granules are usually not apparent. When thrombocytes are more round, they can be difficult to differentiate from lymphocytes. Like mammalian platelets, thrombocytes, when activated, appear in clumps on blood films. Thrombocyte counts are much lower in nonmammalian species than they are in mammals. In most avian species, thrombocyte counts range between  $20 \times 10^3/\mu\text{L}$  and  $30 \times 10^3/\mu\text{L}$ .

As in other blood cells, glucose is the major energy source for platelets. In contrast to anucleated erythrocytes, platelets have mitochondria and, consequently, the Krebs cycle and oxidative phosphorylation; but little of the pyruvate produced by glycolysis is metabolized through the Krebs cycle. Biochemically, platelets have often been compared with white skeletal muscle. They have active anaerobic glycolysis and synthesize and utilize large amounts of glycogen. Platelets have a dense tubular system that is analogous to the calcium ion ( $\text{Ca}^{++}$ )–sequestering sarcoplasmic reticulum present in skeletal muscle (Fig. 6-1). Most domestic animal species have a well-developed canalicular system that is continuous with the surface membrane. Microtubules and microfilaments are present and composed of a variety of contractile proteins including actin, myosin, and related proteins. Microtubule coils help maintain the discoid shape of resting platelets. A major mediator of energy utilization in platelets is an actomyosin-like adenosine triphosphatase that is dependent on  $\text{Ca}^{++}$  and magnesium ions. Changes in actin filament conformation and organization of associated proteins are required for platelet shape change, spreading, aggregation, secretion, and clot retraction.

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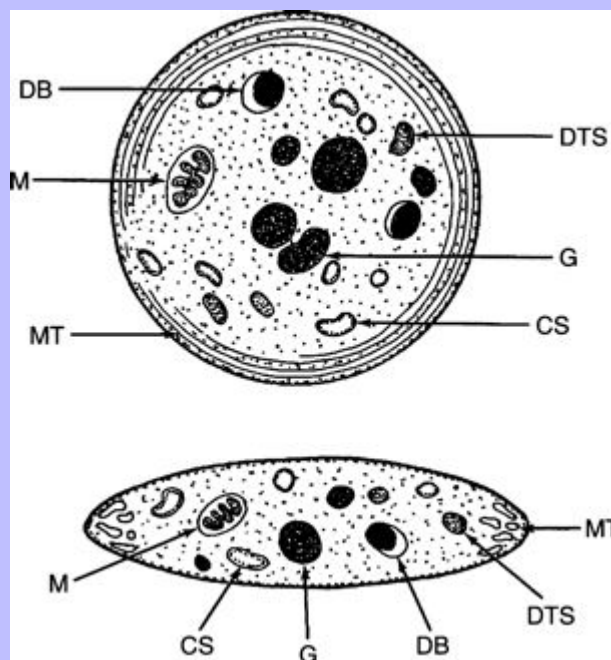
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Adenine nucleotides are present in approximately equal amounts in metabolic and storage pools. As in other cells, adenosine triphosphate (ATP) accounts for most of the adenine nucleotide in the metabolic pool. The ATP in this pool provides energy for cell functions. In addition to energy needed for normal homeostatic processes, platelets expend a large amount of energy during aggregation and the release reaction, which is discussed later. Approximately 50% of the adenine nucleotides are present in platelet-dense bodies or  $\delta$ -granules (storage pool). In

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In addition to ATP, these dense bodies contain considerable adenosine diphosphate (ADP),  $\text{Ca}^{++}$ , magnesium ions, and serotonin, which are secreted outside the platelets during the release reaction (degranulation). Other granules ( $\alpha$ -granules) are also secreted when platelets are activated. Some of the contents of these  $\alpha$ -granules are synthesized by megakaryocytes, and others are taken up from the plasma.  $\alpha$ -Granule contents vary by species. Contents that may be present include adhesive proteins (von Willebrand factor [vWF], fibrinogen, fibronectin, thrombospondin), coagulation factors V and XI, fibrinolytic inhibitors (plasminogen activator inhibitor,  $\alpha_2$ -plasmin inhibitor), chemokines (platelet factor 4 and  $\beta$ -thromboglobulin), P-selectin, glycoprotein (GP) receptors GPIb (CD42b-c) and GPIIb/IIIa, (CD41/CD61), and other components including leukotactic, mitogenic, and vascular permeability factors. Platelets also contain lysozyme granules that contain hydrolytic enzymes.

Fig. 6-1



Platelet ultrastructure. DB, Dense bodies; M, mitochondria; MT, microtubules; DTS, dense tubular system; G, granules; CS, canalicular system.

On their surfaces, platelets express various glycoprotein molecules that are needed for normal adhesion (platelet to extracellular matrix) and aggregation (platelet-to-platelet binding) to occur. The glycoprotein complex GPIb/IX/V (CD42a-d) is especially important for the adhesion of platelets to vWF that is bound to the subendothelial matrix. The GPIIb/IIIa complex ( $\alpha_{IIb}\beta_3$  integrin) is essential for normal platelet aggregation, which is mediated largely by fibrinogen.

Platelets have three primary functions in hemostasis. First, a platelet plug is formed at the site of vessel injury. Formation of a platelet plug alone is sufficient to stop bleeding from a small vessel. Second, platelet activation results in the translocation of negatively charged phospholipids (primarily phosphatidylserine) from inner surfaces to outer surfaces of platelets. These aminophospholipids bind certain coagulation factors in close proximity, thereby accelerating coagulation. Finally, the presence of platelets helps maintain normal vascular integrity in

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some way. Vascular endothelium is thin and more prone to disruption in animals with low platelet counts (thrombocytopenia).

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## 7.2 PRIMARY HEMOSTASIS

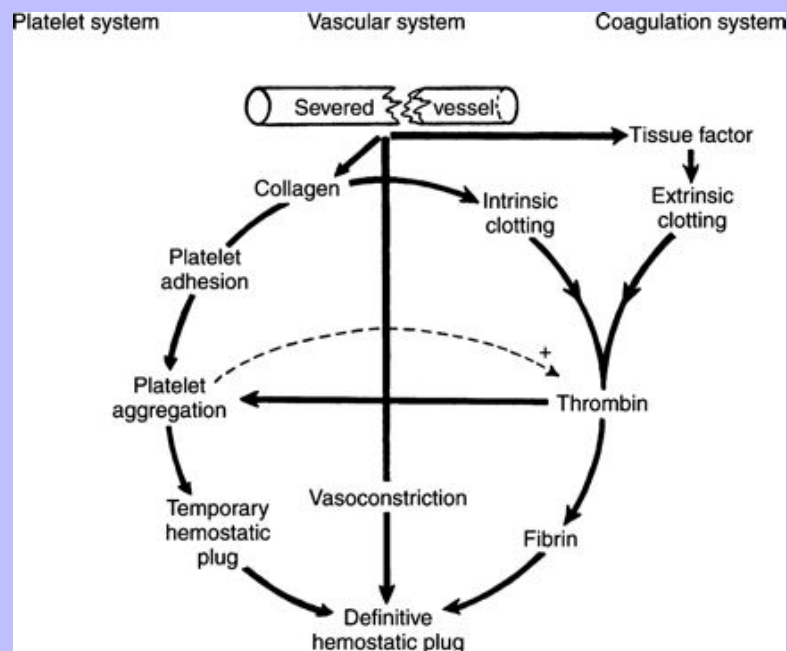
### 7.2.1 Vascular Phase

Primary hemostasis consists of a vascular and a platelet phase (Fig. 6-2). After the severing of vessels, a reflex vasoconstriction temporarily retards blood flow, allowing time for initiation of platelet plug formation and coagulation. The damage or removal of endothelial cells exposes the subcellular matrix, resulting in platelet adhesion and activation, as well as the activation of intrinsic coagulation. Endothelial cells and other damaged tissues release ADP and tissue factor (TF) (tissue thromboplastin), which promote platelet aggregation and extrinsic coagulation, respectively.

### 7.2.2 Platelet Phase

In response to vessel wall injury or exposure to foreign surfaces, platelets rapidly undergo the processes of adhesion, shape change, secretion, and aggregation through a complex series of coordinated processes that culminate in the formation of a precisely located platelet plug. Because of the rapidity of intracellular responses and synergy between secondary messenger systems, it is not possible to organize the various effector systems into a clearly defined temporal sequence. Reciprocal activation of effector systems also makes it difficult to define a clear sequence of events.

Fig. 6-2



Overview of primary hemostasis.

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7.2.2.1

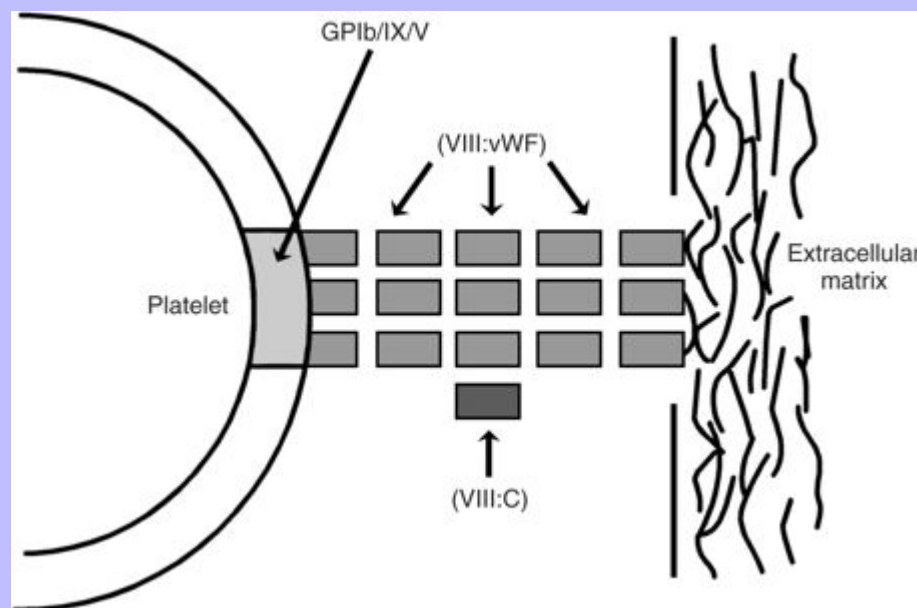
### Platelet Adhesion

Optimal platelet adhesion requires binding of platelet surface glycoprotein GPIb of the leucine-rich GPIb/IX/V complex to vWF molecules. This GPIb/IX/V glycoprotein complex does not bind soluble vWF: it binds only immobilized vWF molecules that have been deposited in the extracellular matrix. vWF in the extracellular matrix is primarily associated with collagen microfibrils (Fig. 6-3). vWF is a component of the factor VIII macromolecular complex. It is a large disulfide-bonded tetramer (molecular weight 850,000 d) that circulates as large multimers (8 to  $12 \times 10^6$  d) in blood. vWF multimers bind to factor VIII coagulant (VIII:C), a smaller protein (molecular weight 250,000 d) that functions as a procoagulant in the intrinsic coagulation system. Binding of factor VIII:C to vWF appears to prolong the circulation time of factor VIII:C. These two factors are controlled by different genes and are synthesized independently. vWF, also referred to as *factor VIII:R (related antigen)*, is encoded by an autosomal gene and synthesized by endothelial cells and megakaryocytes (in some species). Factor VIII:C, also referred to as *factor VIII:AHF (antihemophilic factor)*, is an X-linked gene product that is synthesized by endothelial cells.

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Fig. 6-3



The von Willebrand factor (vWF) component of the factor VIII complex (*VIII:vWF*) is needed for optimal binding of platelets to subendothelial matrix. vWF binds to a glycoprotein complex GPIb/IX/V on the platelet surface. *VIII:C*, Coagulant component of the factor VIII complex.

In addition to its presence in the circulation, vWF is found in platelet granules in some species (dog platelets contain little vWF) and is bound to the extracellular matrix of the vascular subendothelium, where it is secreted by endothelial cells. Platelets do not bind to circulating vWF. It appears that the conformation of vWF changes on binding to extracellular matrix components so that vWF readily binds to the GPIb receptor

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of platelets, especially when shear force is applied, as occurs when flowing blood is exposed to the subendothelial surface.

7.2.2.2

### Platelet Activation

The adhesion of platelets to the extracellular matrix and binding of collagen and other strong agonists (stimulators), such as high concentrations of thrombin and thromboxane  $A_2$  ( $TxA_2$ ), result in the activation of phospholipase C in the platelet membrane (Fig. 6-4). Phospholipase C cleaves a unique membrane phospholipid phosphatidylinositol 4,5-bisphosphate into inositol triphosphate and diacylglycerol. Inositol triphosphate binds to a receptor in the dense tubular system causing the release of intracellular  $Ca^{++}$ , and diacylglycerol potentiates the activation of protein kinase C. These reactions stimulate platelets in various ways and result in shape change, secretion, and aggregation.

Agonists such as ADP, serotonin, and low concentrations of thrombin activate phospholipase  $A_2$ , which stimulates the hydrolysis of phospholipids (especially phosphatidylcholine), causing the release of arachidonic acid. Arachidonic acid is subsequently metabolized to  $TxA_2$  by the cyclooxygenase enzyme pathway (Fig. 6-5). Phospholipase  $A_2$  (in conjunction with an acetyltransferase) is also involved in generating platelet-activating factor (PAF) (1-O-alkyl-2-O-acetyl sn-glycerol-3-phosphoryl choline), another agonist causing platelet aggregation. PAF can be produced not only by activated platelets but also by activated endothelial cells and leukocytes.

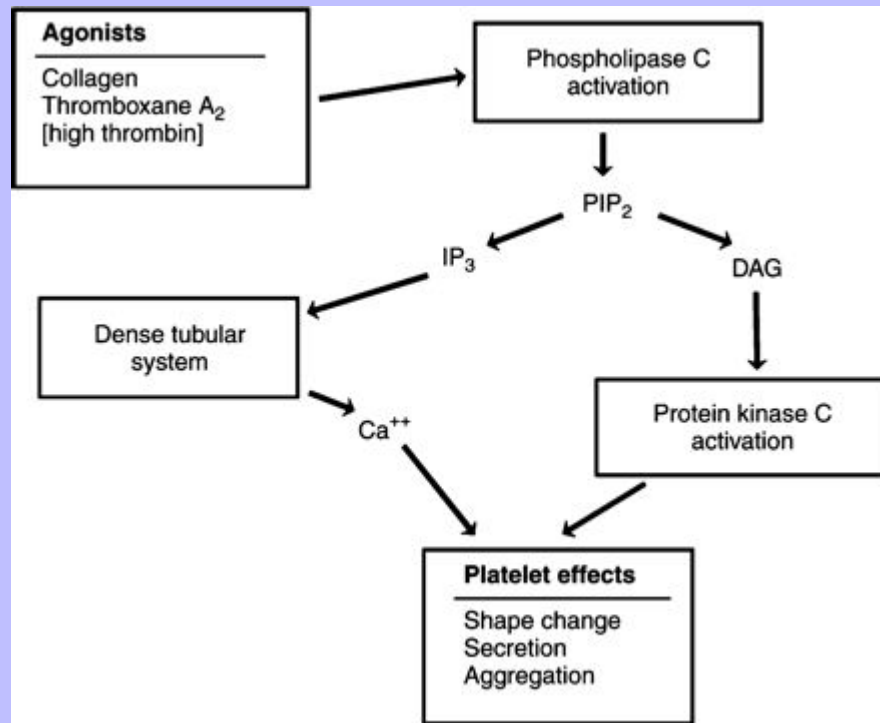
Epinephrine does not function as an agonist by itself, but it potentiates platelet activation and secretion induced by other agonists. Platelets from some dogs do not respond to  $TxA_2$  in vitro because of impaired  $TxA_2$ -G protein coupling, but this defect is normalized by pretreatment with epinephrine.

Platelet activation is inhibited by prostacyclin. When stimulated by thrombin, endothelial cells produce prostacyclin as a product of arachidonic acid metabolism. This antagonist has an antiaggregatory effect by stimulating cyclic adenosine monophosphate synthesis in platelets. (Agonists tend to counteract this effect by lowering platelet cyclic adenosine monophosphate concentrations). Prostacyclin also functions as a vasodilator. Activated endothelial cells also appear to suppress platelet reactivity by increasing the rate of nitric oxide synthesis. Nitric oxide appears to inhibit human platelet adhesiveness better than prostacyclin does.

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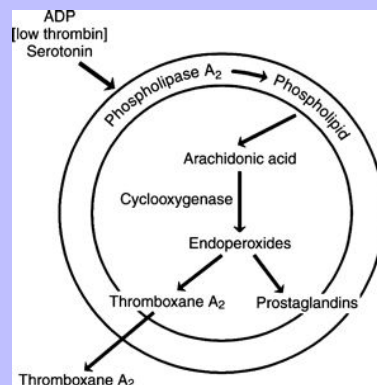
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Fig. 6-4



Activation of phospholipase C and the resultant platelet effects. *PIP<sub>2</sub>*, Phospholipid phosphatidylinositol 4,5-bisphosphate; *IP<sub>3</sub>*, inositol triphosphate; *DAG*, diacylglycerol;  $\text{Ca}^{++}$ , calcium ions.

Fig. 6-5



Activation of phospholipase A<sub>2</sub> and the generation of thromboxane A<sub>2</sub>.

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7.2.2.3

## Shape Change

The discoid shape of resting platelets is maintained by circumferential bundles of microtubules beneath the platelet membrane and an extensive network of short actin filaments, forming a membrane skeleton. After binding to vWF and extracellular matrix components, platelets are activated, the microtubular coils reorganize into linear arrays, and actin polymerization increases, resulting in platelet “spreading” with the formation of filopodia. Negatively charged phospholipids and glycoprotein receptors are also exposed on the surface. Platelets have surface receptors, which are mostly  $\beta_1$ -integrins, for various subendothelial components including collagen, fibronectin, laminin, and thrombospondin. The collagen receptor ( $\alpha_2\beta_1$  integrin) functions for adhesion and also acts as an agonist receptor.

7.2.2.4

## Platelet Secretion

Platelet secretion (release or degranulation) requires energy-dependent contractile mechanisms. Dense bodies and  $\alpha$ -granules are crushed together (fusion and dissolution) by a surrounding web of microtubules and microfilaments. The contents of dense bodies and granules are discharged into the open canalicular system that is continuous with the platelet surface. Cattle platelets and elephant platelets have a minimal canalicular system; granules and dense bodies primarily discharge their contents by fusing with the external platelet membrane. Contraction of individual platelets and the platelet aggregate facilitates the discharge of material into surrounding plasma. ADP, serotonin, and calcium released from dense bodies promote platelet aggregation.

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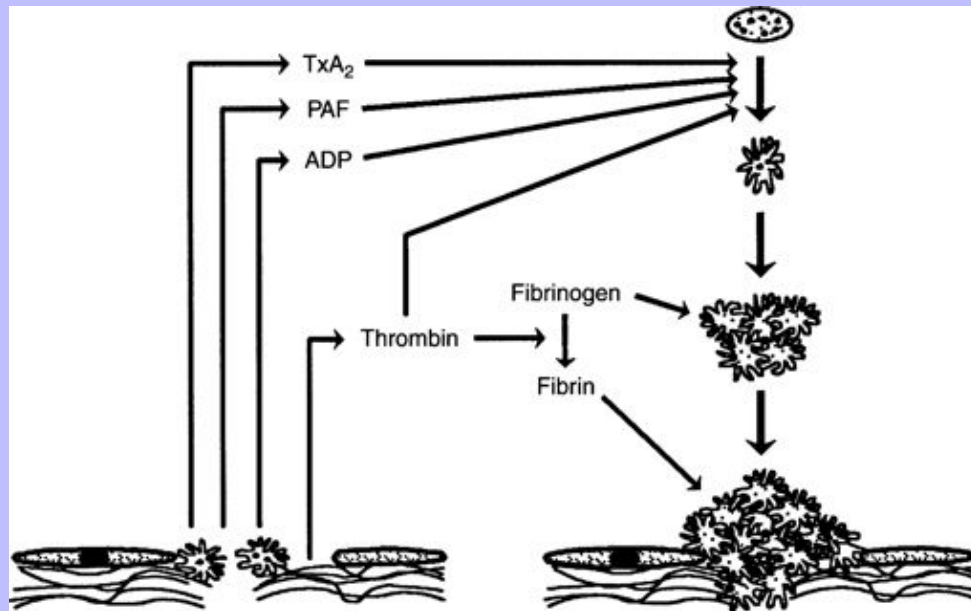
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7.2.2.5

## Platelet Aggregation

ADP,  $\text{TxA}_2$ , thrombin (a product of coagulation), and PAF are consistently important agonists that promote platelet aggregation. Serotonin is also an important agonist in some species. Optimal platelet aggregation requires fibrinogen and  $\text{Ca}^{++}$ . The actions of these agonists result in the exposure or activation of GPIIb/IIIa, a  $\beta_3$ -integrin platelet surface receptor that binds to fibrinogen (Fig. 6-6). Aggregation occurs when symmetric fibrinogen molecules bind to exposed receptors on adjacent platelets. vWF also promotes platelet aggregation when shear forces in flowing blood are large. The resultant platelet plug may be sufficient to stop bleeding from small vessels. As discussed later, the bleeding time test, which measures the time needed to form the platelet plug, is dependent on platelet numbers and function. Avian thrombocytes differ from mammalian platelets in that ADP is not secreted after platelet activation and ADP is not an important agonist for platelet aggregation. Serotonin appears to be an important agonist for avian thrombocytes.

Fig. 6-6



Primary factors involved in platelet aggregation.  $TxA_2$ , Thromboxane  $A_2$ ;  $PAF$ , platelet-activating factor;  $ADP$ , adenosine diphosphate.

### 7.3 SECONDARY HEMOSTASIS

#### 7.3.1 Overview

Secondary hemostasis consists of coagulation and consolidation of the temporary hemostatic platelet plug into a definitive hemostatic plug. Coagulation is an enzymatic process involving the conversion of proenzymes to active enzymes. Some activated coagulation factors are themselves enzymes, and others combine in physical complexes to generate specific enzymatic activities. A cascade effect of enzymatic activation results in an amplification of the original stimulus (Fig. 6-7).  $Ca^{++}$  are required for multiple reactions in coagulation as shown in Figure 6-7. The final product of coagulation is the formation of cross-linked fibrin strands around and, to a lesser extent, through the platelet plug, making it stronger and decreasing the likelihood that rebleeding will occur.

Coagulation factors have been given one or more names, and each has been assigned a Roman numeral.

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Fibrinogen (factor I), prothrombin (factor II), TF (factor III), and  $Ca^{++}$  (factor IV) are usually referred to by their names. Other factors are more often referred to by their numbers. There is no factor VI. All factors except  $Ca^{++}$  are proteins, most of which are synthesized in the liver. All factors except TF are normally present in the circulation.

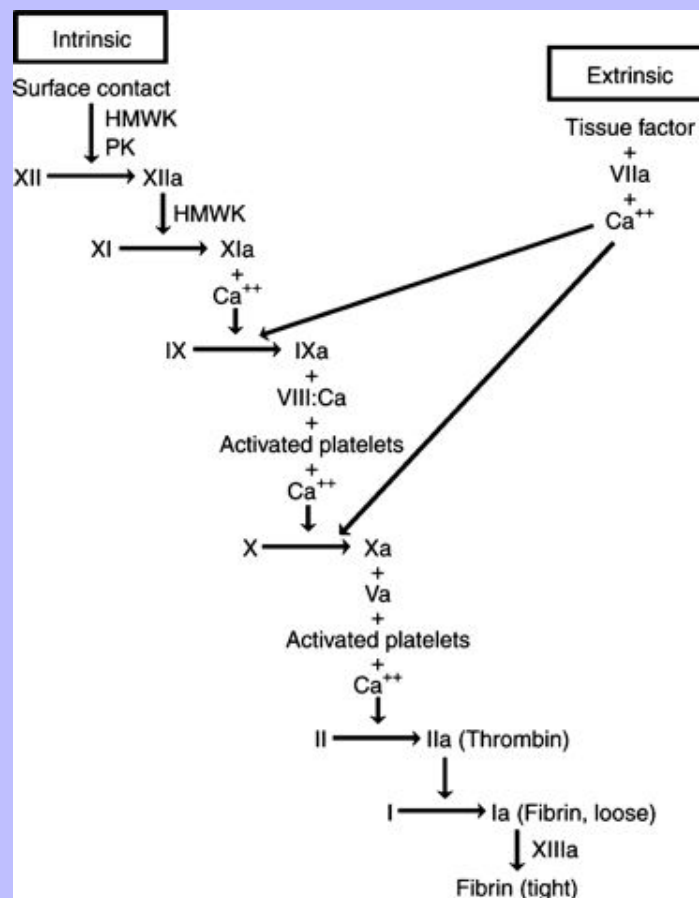
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Vitamin K is required for synthesis of functional factors II, VII, IX, and X. After synthesis of the protein molecules in the liver, a vitamin K-dependent carboxylation of glutamic acid residues on these molecules is required for them to bind  $\text{Ca}^{++}$  and become functional.

Coagulation can be activated by two different mechanisms in vitro: (1) binding of factor VII to TF extracted from tissues (extrinsic pathway) or (2) binding of factor XII to surfaces, resulting in “contact activation” (intrinsic pathway). Factor XII can be activated by binding to a wide variety of surfaces including collagen, basement membranes, skin, and many foreign surfaces including glass. These two activation pathways share a common pathway that generates thrombin. Thrombin not only converts fibrinogen to fibrin but also activates factors V, VIII, XI, and XIII. In vivo activation of coagulation is somewhat different, especially with regard to intrinsic coagulation, as discussed subsequently.

Fig. 6-7

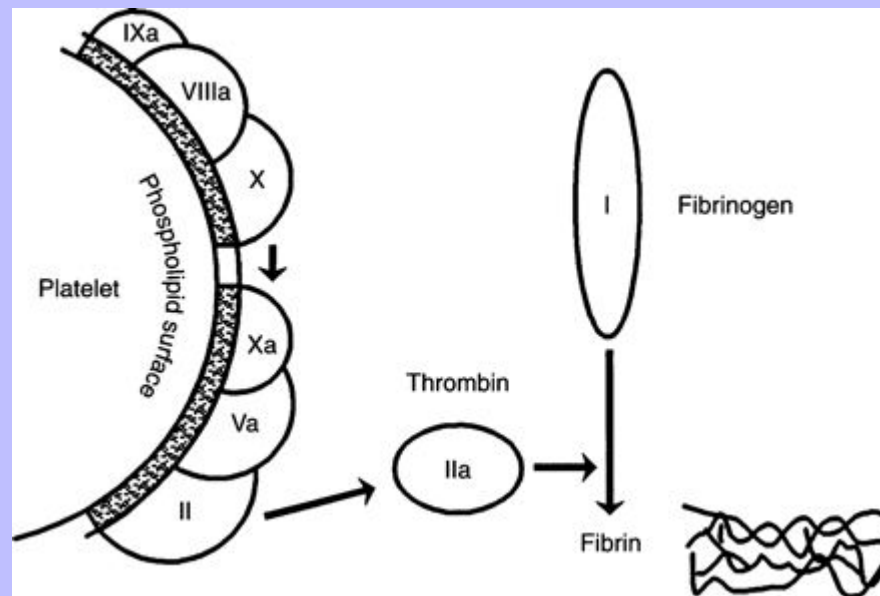


The coagulation cascade. *HMWK*, High-molecular-weight kininogen; *PK*, prekallikrein; *VIII:C*, coagulant component of the factor VIII complex;  $\text{Ca}^{++}$ , calcium ions. Roman numerals refer to coagulant factors with these numbers, and an associated *a* indicates the factor is activated. Activated platelets express negatively charged phospholipids (primarily phosphatidylserine) on their surfaces.

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The activation of platelets by agonists results in translocation of negatively charged phospholipids from the internal to the external plasma membrane. These negatively charged aminophospholipids (primarily phosphatidylserine) have previously been termed *platelet factor 3* or *platelet coagulant activity*. The translocation of phosphatidylserine to the surface of activated platelets accelerates coagulation because positively charged  $\text{Ca}^{++}$  bind to the negatively charged phospholipids and the carboxyl groups of coagulation factors. Binding of coagulation factors to platelets not only brings them together to enhance interactions but also helps protect them from inhibitors (Fig. 6-8).

Fig. 6-8



Assembly of coagulation factors on activated platelet surfaces, primarily binding to the phospholipid phosphatidylserine. Roman numerals refer to coagulation factors with these numbers, and an associated *a* indicates the factor is activated.

### 7.3.2 Extrinsic Coagulation Pathway

It appears that coagulation *in vivo* is initiated by TF (tissue thromboplastin) exposed on the surface of cells, especially activated or damaged endothelial cells. TF is a transmembrane glycoprotein with phospholipid-binding sites. It is not detected in undisturbed endothelial cells but is present in the membranes of many other types of cells that are not normally in contact with the circulation. When TF is exposed on the surface of damaged or activated endothelial cells or when blood comes in contact with extravascular cells expressing TF on their surfaces, the exposed TF binds to circulating factor VII. It is unclear how the initial activated factor VII (VIIa) is formed, but low levels of VIIa are reported in the blood of healthy humans. The TF-VIIa complex on the surface of disturbed endothelial cells activates factors IX and X to IXa and Xa, respectively. The TF-VIIa complex and Xa provide positive feedback to activate more VIIa. It appears that the extrinsic system functions *in vivo* to rapidly provide trace amounts of thrombin to activate factors XI, VIII, and V and cause platelets to aggregate, all of which accelerate intrinsic coagulation.

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## 7.3.3 Intrinsic Coagulation Pathway

Contact activation of coagulation occurs when factor XII is bound to a negatively charged surface and interacts with prekallikrein that is also bound to the surface by high-molecular-weight kininogen (HMWK). Active factor XII (XIIa) and kallikrein are generated by reciprocal activation. Surface-bound XIIa activates factor XI that is also bound to the surface by HMWK. Both prekallikrein and factor XI circulate complexed with HMWK. For many years this method of activation of coagulation was considered to be the major initiating pathway of coagulation. However, since humans, cats, and dogs with factor XII deficiency exhibit no hemorrhagic tendencies and some animals (whales, birds, reptiles) naturally lack this factor, it is generally accepted that factor XII is not involved in normal hemostasis.

Factor XI deficiency does result in mild bleeding tendencies. It appears that factor XI binds to activated platelets and is activated *in vivo* by small amounts of thrombin generated after activation of the extrinsic pathway of coagulation. Factor IX is activated by both XIa and the TF-VIIa complex. Factor VIII:C is activated by small amounts of thrombin. Conversion of factor VIII:C to VIII:Ca requires the dissociation of this molecule from vWF. Factor VIII:Ca is not an enzyme, but it functions as a cofactor that combines with the enzyme IXa on activated platelet surfaces to form a complex, sometimes called *tenase*, that activates factor X (see [Fig. 6-8](#)).

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## 7.3.4 Common Coagulation Pathway

Factor V is activated by small amounts of thrombin to factor Va. Factor Va functions as a cofactor for Xa and forms a complex called *prothrombinase* on the surfaces of activated platelets, which converts prothrombin to thrombin. The thrombin formed converts fibrinogen to fibrin monomers that polymerize spontaneously by hydrogen bonding to form unstable noncross-linked fibrin polymers around the platelet plug. The last step in fibrin polymerization involves the formation of covalent cross-links between fibrin monomers. Factor XIII (fibrin-stabilizing factor) is activated by thrombin. The XIIIa formed is a  $\text{Ca}^{++}$ -dependent transglutaminase that catalyzes the formation of covalent bonds between lysine and glutamine residues of different monomers. The cross-linked fibrin formed is an insoluble protein polymer that stabilizes the platelet plug.

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## 7.3.5 Inhibitors of Thrombus Formation

Once a thrombus composed of platelets and fibrin is formed over an area of vascular injury, the coagulation process must be terminated to prevent thrombotic occlusion of normal areas of the vasculature adjacent to the injury. Endothelial cells are especially important in limiting the thrombus formation to the site of injury by various mechanisms. Unstimulated platelets do not adhere to the surface of healthy vascular endothelial cells because these endothelial cells possess thromboresistant properties. Endothelial cells synthesize and release prostacyclin and nitric oxide, which are powerful vasodilators that also inhibit platelet aggregation. Endothelial cells also inhibit platelet function by virtue of an ectoenzyme, CD39, that has adenosine diphosphatase activity, which can degrade ADP released from activated platelets.

Endothelial cells synthesize heparan sulfate proteoglycans, which are tightly associated with the endothelium and can accelerate the inactivation of coagulation factors by antithrombin III. Endothelial cells also express thrombomodulin. This cell surface glycoprotein inhibits thrombin's procoagulant activities and promotes protein C activation by thrombin (discussed later). Tissue factor pathway inhibitor (TFPI) is synthesized by microvascular endothelial cells. Lastly, endothelial cells produce tissue plasminogen activator (tPA), which is integrally involved in fibrinolysis.

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Antithrombin III is the major thrombin inhibitor. It also inhibits other proteases including factors IXa, Xa, XIa, and XIIa. It requires the presence of glycosaminoglycans (e.g., heparan sulfate on cell surfaces or in the intracellular matrix) for optimal activity.

Factor Xa and the TF-VIIa complex are inhibited by the bivalent TFPI, formerly called *the lipoprotein-associated coagulation inhibitor*.  $\alpha_1$ -Protease inhibitor ( $\alpha_1$ -antitrypsin), C1-esterase inhibitor, and  $\alpha_2$ -macroglobulin are less important inhibitors of thrombus formation.

### 7.4 FIBRINOLYSIS

Thrombin binds to a protein receptor (thrombomodulin) on endothelial cells and this 1:1 complex activates protein C, a vitamin K-dependent plasma protein (Fig. 6-9). Thrombin that is bound to thrombomodulin no longer has significant procoagulant activity. Protein C—in the presence of a vitamin K-dependent cofactor (protein S), a phospholipid surface, and  $\text{Ca}^{++}$ —inhibits coagulation by proteolytically degrading factors Va and VIIIa. Protein S also appears to inhibit coagulation independently of protein C by reversible binding to factors Va, VIIIa, and Xa.

Protein C binds to an inhibitor of tPA and increases the release of tPA from endothelial cells. Plasminogen coprecipitates with fibrin as a thrombus forms. Conversion of plasminogen to plasmin by tPA is accelerated in the presence of fibrin (Fig. 6-10). Plasmin is not a highly specific enzyme, but its affinity for fibrin helps limit its action. Plasmin-catalyzed hydrolysis of fibrin results in the formation of fibrin degradation products (FDPs) (fibrin split products) that have antihemostatic properties.

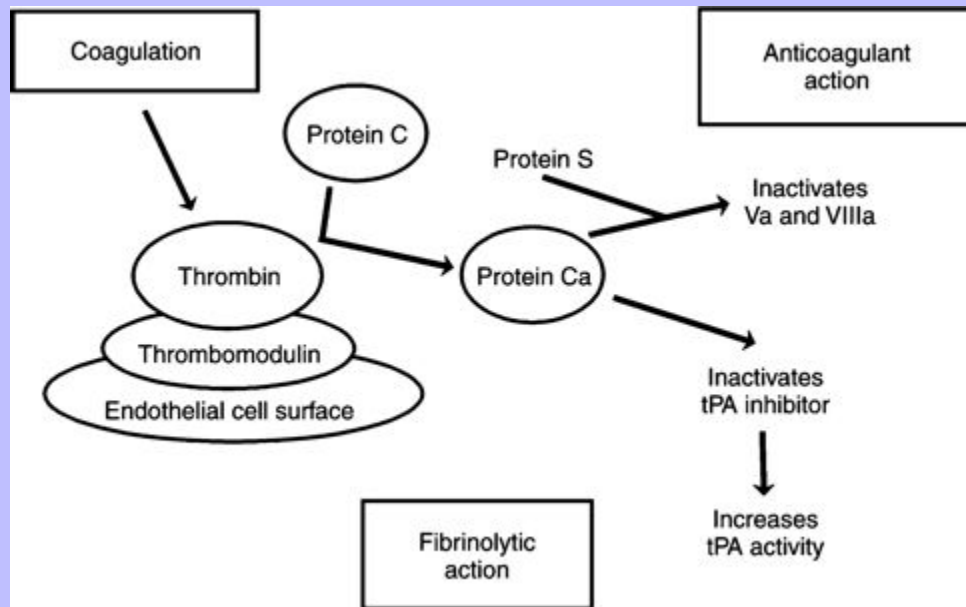
Inhibitors of fibrinolysis also occur in plasma. Endothelial cells produce plasminogen activator inhibitor that inhibits tPA.  $\alpha_2$ -Antiplasmin inhibits free plasmin, but plasmin bound to fibrin is protected.  $\alpha_2$ -Macroglobulin inhibits protein C and may inhibit plasmin to some extent. It also inhibits some activated coagulation factors.

Fibrinolysis occurs more readily in capillaries than in the systemic circulation. A much higher density of endothelial cells occurs in capillaries. It is estimated that capillaries comprise more than 99% of the endothelial surface area of the body. Consequently, thrombomodulin-mediated protein C activation and thrombin clearance are greater in capillaries than in large vessels. In addition, tPA release is greater in capillaries and there may be less antiplasmin available to inhibit fibrinolysis. Rapid fibrinolysis of thrombi in large vessels could be life-threatening, but fibrinolysis may be important in maintaining the integrity of capillary beds.

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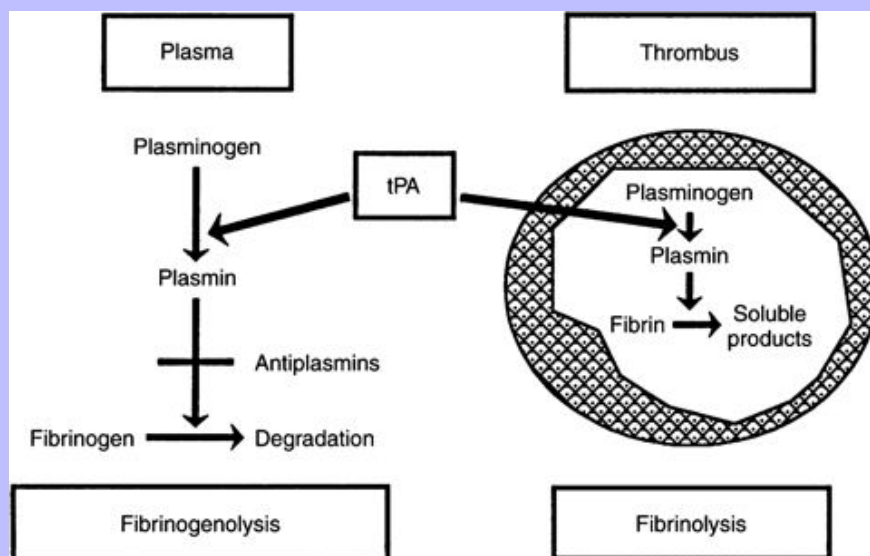
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Fig. 6-9



Actions involving protein C. *tPA*, Tissue plasminogen activator. Roman numerals refer to coagulation factors with these numbers, and an associated *a* indicates the factor is activated.

Fig. 6-10



Actions of tissue plasminogen activator (*tPA*) and plasmin.

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## 7.5 ANTICOAGULANTS

Coagulation is prevented in blood samples by using either  $\text{Ca}^{++}$  chelators (ethylenediaminetetraacetic acid [EDTA] and citrate) or heparin in blood collection tubes. EDTA is the preferred anticoagulant for complete blood count determinations in most species. Minimal sample dilution occurs after mixing with EDTA, and blood films prepared with this anticoagulant exhibit optimal staining with routine blood stains. Unfortunately, blood from some birds and reptiles hemolyzes when it is collected with EDTA. In those species, heparin is usually used as the anticoagulant. The disadvantage of using heparin is that leukocytes do not stain as well and platelets usually clump more than in blood collected with EDTA.

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Citrate is the preferred anticoagulant for collecting plasma for coagulation tests and for collecting platelets for platelet function tests. Samples collected in citrate solution are diluted by 10%. If platelet counts are done, they must be corrected for this dilution. Citrate is also the anticoagulant typically used in blood collection and storage for transfusions.

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The binding of heparin to antithrombin III greatly accelerates the inhibition of thrombin by antithrombin III, thereby inhibiting coagulation. Coagulation factors IXa and Xa and the VIIa-TF complex also appear to be inhibited by the antithrombin III–heparin complex. Heparin is used as an anticoagulant for complete blood counts in blood from species in which EDTA causes hemolysis. Lithium heparin is used as an anticoagulant when plasma (rather than serum) is used for clinical chemistry profiles. Heparin is often added to isotonic salt solutions used to flush intravenous lines and may be injected to inhibit blood coagulation in vivo.

## 7.6 SCREENING TESTS FOR HEMOSTATIC DISORDERS

No single diagnostic test evaluates all hemostatic components. Consequently, several hemostatic tests are usually done to determine the nature of a hemostatic disorder.

### 7.6.1 Platelet Count

Stained blood films should be examined each time platelet counts are done to verify that low platelet counts, determined manually or by machine, are valid. The presence of platelet clumps can result in erroneously low counts. Factors used to estimate platelet numbers vary depending on the microscope used, method of blood film preparation, and the area of the film examined; however, the following formula generally provides a reasonable platelet estimate.

Platelets per microliter  
= Number of platelets per 100X oil field  $\times$  20,000

Cat platelets are larger than those of other domestic animals; consequently, it is not possible for impedance counters (such as the Coulter Counter S+4 and the Abbott Cell-Dyne 3500) to accurately separate cat platelets from erythrocytes by size. Cell counters that count platelets by using laser flow cytometry (such as the Bayer Advia 120) are able to count cat platelets more accurately in whole blood. Unfortunately, platelet aggregates form readily during blood collection in cats; as a result, spuriously low automated platelet counts will often be present. Anticoagulants with platelet inhibitors added, which would decrease platelet aggregate formation, may become available.

Platelet counts in healthy greyhounds and Cavalier King Charles spaniels are generally lower than those measured in other dog breeds. The lower limit of the reference interval for platelet counts in healthy

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greyhounds is reported to be about  $70 \times 10^3/\mu\text{L}$  when determined with automated impedance counters. Somewhat higher platelet counts would likely be measured manually. The lower limit of the reference interval for platelet counts in Cavalier King Charles spaniels is difficult to determine with certainty, because there is a high incidence of an asymptomatic inherited thrombocytopenia with macroplatelets in this breed. The lower limit of the reference interval is believed to be about  $100 \times 10^3/\mu\text{L}$ , because platelet size is typically normal in animals with platelet counts greater than  $100 \times 10^3/\mu\text{L}$ . Platelet counts determined with impedance counters are erroneously low in Cavalier King Charles spaniels with macroplatelets, because these counters cannot differentiate large platelets from small erythrocytes.

## 7.6.2 Mean Platelet Volume

The mean platelet volume (MPV) is the average volume of a single platelet recorded in femtoliters. Impedance cell counters can accurately determine the MPV in whole blood from dogs and horses, but not in whole blood from cats. Cell counters that count and size platelets by means of laser flow cytometry may be able to accurately measure the MPV in whole blood of cats, but platelet aggregates form readily during blood collection in cats, resulting in spuriously high MPV values.

Before the platelet count and MPV from a blood sample are considered valid, cell counters are used to determine whether the size distribution of the platelets approximates the histogram normally expected for platelets. If the histogram is abnormal in shape or if insufficient numbers of platelets are present for accurate histogram construction (very low platelet counts), the values will not be reported. Consequently, whole blood platelet counts and MPV values cannot be determined for every sample. Unfortunately, MPV values are often not reported for animals with thrombocytopenia, for which they might provide useful information.

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Within the normal ranges of platelet counts and MPVs, there is an inverse correlation between platelet count and MPV in some species. Results of studies on the effects of anticoagulants and storage conditions on MPV are inconclusive. MPV values may be slightly higher when blood is collected with EDTA versus citrate as an anticoagulant, and increases in MPV may be more likely when blood is stored at  $5^\circ\text{C}$  versus room temperature. A high MPV value suggests that enhanced thrombopoiesis is present, but MPV can also be high in animals with myelodysplastic disorders. Cavalier King Charles spaniels with an inherited thrombocytopenia may have higher MPV values because of the occurrence of a population of macroplatelets. A normal MPV value does not rule out enhanced thrombopoiesis. The presence of small platelet aggregations in blood samples can result in an artificially increased MPV value. Decreased MPV (presence of microthrombocytes) has been associated with immune-mediated thrombocytopenia in dogs and humans as a result of platelet fragmentation. After appropriate therapy, MPV may increase above normal in individuals with immune-mediated thrombocytopenia. MPVs have been reported to be slightly higher in cats with hyperthyroidism and slightly lower in dogs with hypothyroidism than in euthyroid animals. Dogs with phosphofructokinase deficiency of erythrocytes and skeletal muscle have mildly increased MPVs with normal platelet counts.

## 7.6.3 Bleeding Time

The bleeding time is a simple but crude hemostatic test. It is prolonged in animals with platelet abnormalities. Since bleeding time is expected to be prolonged in animals with low platelet counts, this test provides no additional information about animals already known to have severe thrombocytopenia. The buccal mucosal bleeding time is determined to evaluate platelet function in animals with normal or near-normal platelet counts. After penetration of the buccal mucosa with a lancet, the site should bleed freely. If left undisturbed, bleeding will usually stop in less than 4 minutes in healthy dogs and in less than 3 minutes in cats.

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Bleeding times determined by toenail clip (cuticle bleeding time) in anesthetized dogs and cats not only evaluate platelet function but may also detect severe coagulation defects because the vessel injury caused by this technique is more substantial than that produced in the test for buccal mucosal bleeding time. The cuticle bleeding time is difficult to standardize and lacks repeatability.

## 7.6.4 Activated Clotting Time

The activated clotting time (ACT) test evaluates the intrinsic and common coagulation pathways ([Fig. 6-11](#)). It must be done in close proximity to the animal being evaluated. The ACT requires a special collection tube containing siliceous earth (Product No. 366522; Becton, Dickinson and Company) and a method to maintain collection tubes at 37°C. Blood is collected into prewarmed tubes and maintained at 37°C until clotting has occurred. The ACT is usually less than 200 seconds in horses, less than 165 seconds in cats, and less than 125 seconds in dogs.

## 7.6.5 Activated Partial Thromboplastin Time

The activated partial thromboplastin time (APTT) test is also used to evaluate the intrinsic and common pathways. APTT is measured with plasma prepared from blood collected with 3.2% sodium citrate as the anticoagulant in a 9:1 mixture. Samples should be kept cool, and assays should be done within 30 minutes of collection. APTT from a healthy control animal should be measured at the same time. The APTT is likely prolonged if the patient's time is 30% or more longer than the control's time. The reference interval will depend on the method used. The APTT is artifactually prolonged if the plasma to anticoagulant ratio is inappropriately low, as occurs if insufficient blood is collected into a vacuum tube containing premeasured citrate solution or if erythrocytosis is present (e.g., if severe dehydration is present).

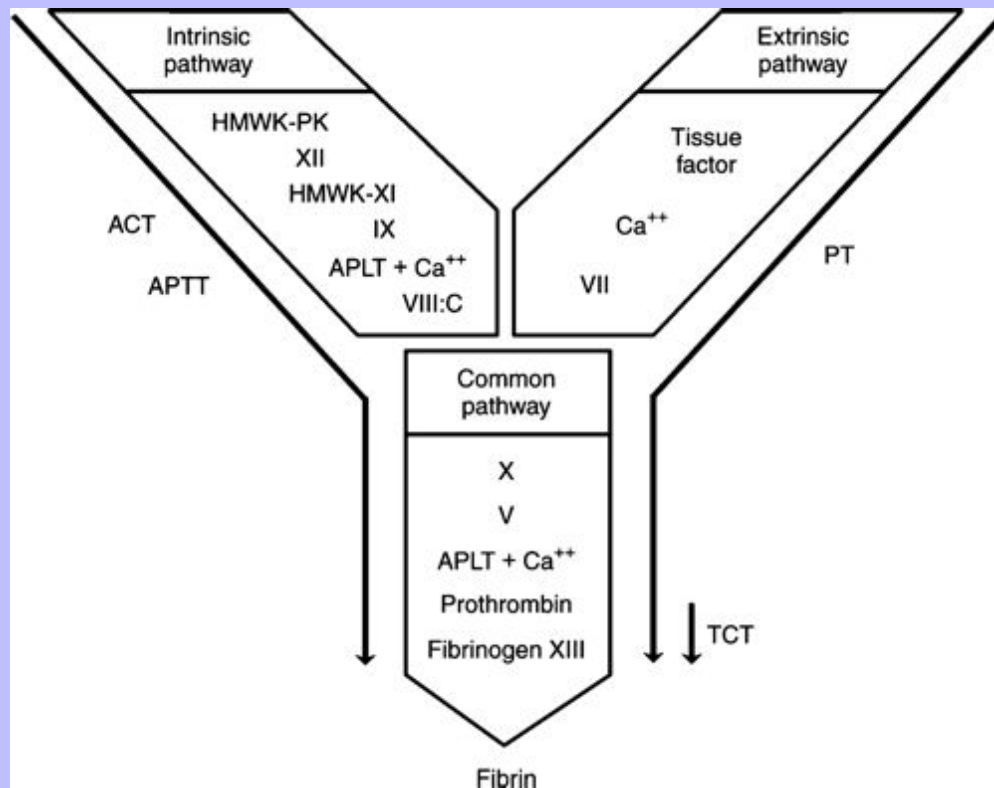
## 7.6.6 Prothrombin Time

The prothrombin time (PT) test is used to evaluate the extrinsic and common pathways (see [Fig. 6-11](#)). It also requires plasma prepared from blood collected with citrate as the anticoagulant. Samples should be kept cool, and assays should be done within 30 minutes of collection. PT of a healthy control animal should be measured at the same time. The PT is likely prolonged if the patient's time is 30% or more longer than the control's time. The reference interval will depend on the method used.

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Fig. 6-11



Parts of the coagulation cascade evaluated by using activated clotting time (*ACT*), activated partial thromboplastin time (*APTT*), prothrombin time (*PT*), and thrombin clotting time (*TCT*) tests. *HMWK*, High-molecular-weight kininogen; *PK*, prekallikrein;  $\text{Ca}^{++}$ , calcium ions; *APLT*, activated platelets; *VIII:C*, coagulant component of the factor VIII complex.

### 7.6.7 Thrombin Clotting Time

The thrombin clotting time (TCT) test is an indicator of quantitative and qualitative fibrinogen disorders. Citrated plasma is used in the TCT. Samples should be kept cool, and assays should be done within 30 minutes of collection. A healthy control animal's TCT should be measured at the same time for comparison with the patient's TCT. The reference interval will depend on the method used.

### 7.6.8 Fibrinogen

The heat precipitation test is a practical test for the estimation of fibrinogen values, but it is not sensitive enough to differentiate low-normal from low fibrinogen values when determinations are made by subtraction of total protein values measured with a refractometer. More accurate values can be obtained by ocular micrometry,

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but somewhat more time is required and a microscope with a calibrated ocular micrometer is needed. Fibrinogen is most accurately measured by using a coagulation-based assay.

## 7.6.9 Fibrin Degradation Products

The fibrin degradation product (FDP) test provides evidence of fibrinolysis in vivo. FDPs produced by fibrinolysis have antihemostatic properties that promote hemorrhage, which may occur as a sequela to disseminated intravascular coagulation (DIC). Antibody-based kits for measurement of FDPs in human plasma and serum are commercially available. Fortunately, several assays have sufficient cross-reactivity to be used for domestic animals, but assay results can vary depending on the kit used. Positive FDP test results occur in animals with fibrinolysis (initiated by DIC) and animals with fibrinogenolysis (e.g., Eastern diamondback rattlesnake envenomation). Inexplicably, some dogs with anticoagulant rodenticide toxicity have been reported to have positive FDP test results when an assay of serum is done. These results need to be confirmed by using FDP assays of plasma. Slightly increased FDP values have been associated with exercise, anxiety, and stress in humans. False-positive results can be caused by improper collection and handling of samples.

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When plasmin cleaves soluble fibrin (or fibrinogen), FDP fragments X, Y, D, and E are produced. When plasmin cleaves cross-linked fibrin, different degradation products are produced. D-dimer is the smallest breakdown product produced by the action of plasmin on cross-linked fibrin. The D-dimer tests utilize monoclonal antibodies against a human D-dimer epitope. A positive D-dimer test result indicates the presence of fibrinolysis. Traditional FDP assays cannot distinguish between fibrinolysis and fibrinogenolysis. More studies are needed, but initial findings suggest that results of the D-dimer test and other FDP tests are similar in animals.

## 7.7 SPECIALIZED TESTS FOR HEMOSTATIC DISORDERS

### 7.7.1 von Willebrand Factor (Factor VIII–Related Antigen)

vWF is a glycoprotein that is composed of multimers of various molecular weights. This factor is required for normal platelet adhesion; consequently, it is assayed when a platelet function defect is suspected. Inherited vWF deficiency (von Willebrand disease) is especially common in certain dog breeds (up to 70% of Doberman pinschers). Consequently, this factor may be measured before surgery or breeding of dogs from breeds in which the disease is prevalent. Some investigators have indicated that hypothyroidism may result in decreased vWF concentrations in plasma in dogs, but other investigators have been unable to confirm this relationship. vWF may increase in plasma as an acute-phase protein during inflammation. Increased concentrations have been reported in dogs with liver disease, during parturition, during endotoxemia, and after epinephrine infusion. Interleukin (IL)–11 promotes vWF synthesis in dogs. In contrast, desmopressin (1-deamino-8 $\beta$ -arginine vasopressin) treatment transiently increases vWF concentration in plasma by stimulating the release of vWF stored in endothelial Weibel-Palade bodies.

vWF concentration in plasma is generally quantified by using an enzyme-linked immunosorbent assay (ELISA). There is substantial temporal variation in individual dogs, making identification of carrier animals difficult. Consequently, multiple tests may be necessary to obtain a reliable estimate of vWF concentration. High-molecular-weight vWF multimers are required for normal platelet adhesion. Multimeric distribution of vWF is determined by protein immunoelectrophoresis.

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## 7.7.2 Antithrombin III

Plasma antithrombin III can be measured by means of chromogen assays. It may be decreased in hypercoagulable states (e.g., equine colic), DIC, protein-losing nephropathies, protein-losing enteropathies, and sepsis. Antithrombin III is increased in cats with various disease conditions, suggesting that it behaves as an acute-phase protein in this species.

## 7.7.3 PIVKA (Proteins Induced by Vitamin K Absence or Antagonism)

The PIVKA test (Thrombotest; Accurate Chemical and Scientific Corp, Westbury, NY) is a simple, sensitive coagulation test that was developed to monitor humans treated with warfarin. It is essentially a modified PT test that uses a particular tissue thromboplastin and diluted plasma to result in longer clotting times than the standard PT test. The PIVKA test is not specific for vitamin K deficiency. It may be prolonged with various defects in the extrinsic and/or common pathways. The PIVKA test does not offer any advantage over the PT test in the diagnosis of rodenticide toxicities, because PT is consistently markedly prolonged in these disorders. However, the PIVKA test might be helpful in identifying subtle coagulation abnormalities of the extrinsic or common pathways because PT tests designed for humans may lack sensitivity for the detection of coagulopathies in certain animal species.

## 7.7.4 Reticulated Platelet Count

Reticulated platelets are newly formed platelets that contain increased amounts of RNA. They can be quantified by detecting fluorescence in thiazole orange–stained platelets with a flow cytometer, although this assay is not readily available. Reference intervals vary considerably, depending on the methods and equipment used for the assays. Analogous to the use of reticulocyte counts in determining the cause of an anemia, an increased percentage of reticulated platelets in an animal with thrombocytopenia indicates that the thrombocytopenia resulted from increased platelet destruction or consumption and not from decreased platelet production. In contrast to regenerative anemias, in which increases in absolute reticulocyte counts are expected, absolute reticulated platelet counts (reticulated platelets per microliter) are not typically increased in regenerative thrombocytopenias. This lack of increase in total reticulated platelets in blood may be explained if reticulated platelets are destroyed or consumed at the same rate as nonreticulated platelets. Total reticulated platelet counts may be increased in reactive thrombocytosis, as has been demonstrated in dogs given IL-6 experimentally. The percentage of reticulated platelets is generally within the reference interval in animals with thrombocytopenia resulting from decreased platelet production.

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## 7.7.5 Platelet Function

Specialized platelet function tests are not done in most clinical pathology laboratories but are typically done in hemostasis research laboratories. Platelet aggregation is evaluated after the addition of various agonists (e.g., ADP, thrombin, and collagen). Platelet adhesion can be measured by the retention of platelets in a glass bead column or a filter of standard size.

## 7.7.6 Antiplatelet Antibody

Increased platelet-bound immunoglobulins have been detected by flow cytometry and ELISA techniques in animals, but these tests are not readily available. Direct assays (patient's platelets) are preferred to indirect

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assays (patient's serum and normal canine platelets) because direct assays are more sensitive. Unfortunately, these assays must generally be done within a few hours after blood sample collection. Platelets naturally have some immunoglobulin adsorbed to their surfaces, and the amount of platelet-bound immunoglobulin increases with time after sample collection; consequently, false-positive test results are a significant problem in these assays. Positive test results may occur when immune complexes are adsorbed to platelets, as well as when antiplatelet antibodies are present.

## 7.7.7 Specific Coagulation Factors

Assays for specific coagulation factors are done in a few hemostasis research laboratories. Plasma from humans or animals with known coagulation factor deficiencies is used in these tests.

## 7.8 CLINICAL SIGNS OF HEMOSTATIC DISORDERS

If bleeding is excessive or unexplained, a defect in one or more of the components of hemostasis may be present. The type of hemorrhage observed may give some clue about the nature of the defect(s) present. Diffuse cutaneous or mucosal discoloration, resulting from hemorrhage and edema, suggests the presence of a vascular defect. Petechial and ecchymotic cutaneous and/or mucosal hemorrhages and epistaxis are suggestive of thrombocytopenia. The presence of inherited platelet defects should be suspected in young animals with epistaxis, mucosal bleeding, unexplained petechial and ecchymotic hemorrhages, and excessive bleeding after shedding of teeth or minor trauma. Mucosal hemorrhage, cutaneous bruising, and prolonged bleeding from surgical or traumatic wounds are typically seen in dogs with vWD, but petechiae do not appear to be a sign of vWD in dogs. Spontaneous hematomas and hemarthrosis are more likely to result from coagulation defects than from vascular or platelet abnormalities.

## 7.9 PLATELET DISORDERS

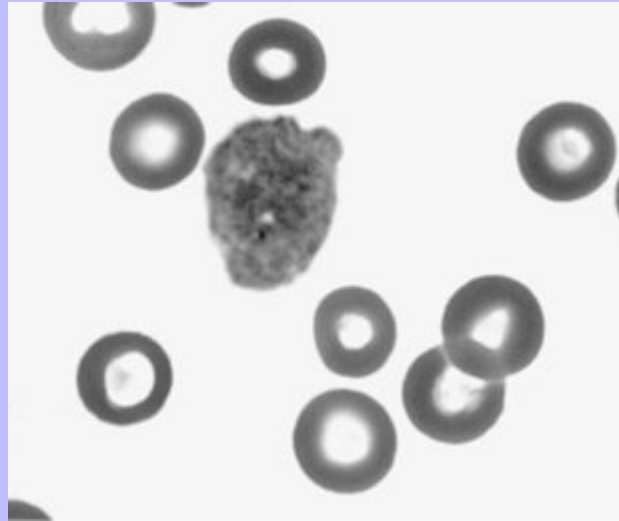
### 7.9.1 Abnormal Platelet Morphology

The diameters of platelets vary depending on the species, with cats having larger platelets than other domestic animals. The presence of large platelets (macroplatelets or megaplatelets) in an animal with thrombocytopenia suggests that enhanced thrombopoiesis is present (Fig. 6-12), but macroplatelets may also be present in animals with thrombocytopenia and myelodysplastic or myeloproliferative disorders. A population of macroplatelets may be seen in some healthy Cavalier King Charles spaniels.

When platelets are activated, their granules are crushed together by a surrounding web of microtubules and microfilaments. This central aggregate of platelet granules may be mistaken for a nucleus. Hypogranular platelets may result from platelet activation and secretion, but they have also been seen in animals with myeloproliferative disorders. Platelet aggregates form after platelet activation in vitro. If degranulation occurs, aggregates may be difficult to recognize, appearing as light blue material on stained blood films. The presence of platelet aggregates should be recorded because the platelet count may be erroneously decreased when aggregates are present.

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Fig. 6-12



A macroplatelet in blood from a dog with thrombocytopenia and enhanced thrombopoiesis.

*Anaplasma platys* (formerly *Ehrlichia platys*) is a rickettsial parasite that specifically infects dog platelets. Morulae appear as tightly packed basophilic clusters of organisms within the cytoplasm of platelets ([Plate 18](#)).

### 7.9.2

#### Thrombocytopenia

Thrombocytopenia denotes decreased blood platelets. Primary causes of thrombocytopenia include decreased production, increased platelet utilization in thrombus formation, and increased destruction. The distinction between these causes is not always clear, and the pathogenesis of thrombocytopenia associated with infectious agents (viral, rickettsial, bacterial, and protozoal) appears to be multifactorial. Less likely causes of thrombocytopenia include sequestration and acute massive external hemorrhage. Bone marrow examination is often indicated in the differential diagnosis of thrombocytopenia, especially in the absence of accompanying coagulation abnormalities.

#### 7.9.2.1

##### Decreased Platelet Production

Myelophthisis, myeloproliferative disorders, and agents that cause aplastic anemia (see [Chapter 5](#)) usually have associated thrombocytopenias. Late stages of infections with *Ehrlichia canis* and possibly other rickettsial diseases can have decreased platelet counts secondary to hypoplastic bone marrow. Most immune-mediated thrombocytopenias result from increased platelet destruction, but an immune-mediated destruction of megakaryocytes can also result in decreased platelet production. Amegakaryocytic thrombocytopenia is rare in dogs and has been described only once in a cat. Mild cyclic thrombocytopenia occurs because of intermittently decreased platelet production in gray collie dogs with inherited cyclic hematopoiesis. The long-term use of a recombinant human thrombopoietin can result in a persistent thrombocytopenia in animals, when antibodies made against the recombinant thrombopoietin also neutralize

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the endogenous thrombopoietin of the species receiving treatment. This subsequent lack of endogenous thrombopoietin results in decreased platelet production.

7.9.2.2

### Increased Platelet Utilization

Increased platelet utilization (consumption) also occurs in association with DIC (discussed later), hemangiosarcoma in dogs, vasculitis, and many other disorders that result in endothelial injury. In addition to vascular injury associated with certain inflammatory conditions, some inflammatory cytokines (most notably PAF) promote platelet aggregation. Thrombocytopenia is often present after rattlesnake envenomation. Components in venom may directly induce platelet activation and aggregation, and aggregation may also occur in response to vessel injury induced by components of venom.

7.9.2.3

### Increased Platelet Destruction

The presence of increased immunoglobulin on the surface of platelets can result in increased phagocytosis of platelets and subsequent thrombocytopenia. Immune-mediated thrombocytopenia can be either primary or secondary. Autoantibodies are directed against platelet-specific epitopes in primary immune-mediated thrombocytopenia. Autoimmune thrombocytopenia (also called *idiopathic thrombocytopenia purpura*) is a common cause of thrombocytopenia in dogs, but it appears to be rare in other species. Secondary immune-mediated thrombocytopenia results from the exposure of hidden or altered antigens on platelet surfaces, the binding of external antigens (e.g., drugs) to platelets, or the adsorption of antigen-antibody complexes to the platelet surface. Secondary immune-mediated thrombocytopenia may occur in association with various drugs, infectious agents, neoplasia, and other immune-mediated disorders, such as systemic lupus erythematosus. Neonatal alloimmune thrombocytopenia has rarely been reported in newborn pigs, horses, and mules after nursing. Analogous to neonatal isoerythrolysis, this disorder occurs when maternal antibodies against paternal epitopes on the surface of neonatal platelets are passively transferred to the neonate in colostrum.

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Intravenous administration of heparin frequently induces mild thrombocytopenia in some horses. The mechanism has not been determined, but heparin-induced thrombocytopenia appears to be immune-mediated in humans. Heparin binds to platelet factor 4 in plasma and antibodies are made against the heparin-platelet factor 4 complex. The antibody-platelet factor 4-heparin complex binds to Fc receptors on the platelet surface and cross-links the receptors. This induces intense platelet activation and platelet aggregation.

Thrombocytopenia can occur with a wide variety of bacterial, viral, protozoal, and fungal infections. *Ehrlichia canis* is a common infectious cause of thrombocytopenia in dogs in the southeastern United States. This agent appears to cause immune-mediated platelet destruction in the acute phase of ehrlichiosis and decreased platelet production in the severe, chronic phase of ehrlichiosis. The cause(s) of thrombocytopenias associated with other infectious agents is not always clear. Vascular injury, adsorption of immune complexes to platelet surfaces, or both may be involved. Viruses are believed to have direct effects on platelets in some viral infections with thrombocytopenia. The administration of granulocyte-monocyte colony-stimulating factor and macrophage colony-stimulating factor to dogs results in shortened platelet life span and thrombocytopenia, apparently by activating the monocyte/macrophage system. Monocyte and/or macrophage activation may be a mechanism of increased platelet destruction observed in a variety of inflammatory diseases in which these endogenous cytokine concentrations in plasma are increased. *Anaplasma platys* is a rickettsial parasite that specifically infects dog platelets and causes a cyclic thrombocytopenia.

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## 7.9.2.4 Sequestration of Platelets

Sequestration of platelets in the body may result in thrombocytopenia. Examples include splenomegaly and hypothermia in various species. Causes of splenomegaly include hereditary hemolytic anemias, immune-mediated diseases, infections, inflammation, splenic congestion, and infiltrative diseases. Increased platelet utilization or destruction also contributes to the development of thrombocytopenia in some of these disorders. When splenomegaly results in increased removal of platelets and/or other blood cells by the spleen, the term *hypersplenism* may be used.

## 7.9.2.5 Massive External Hemorrhage

Because of platelet storage in the spleen, the lungs, or both, acute hemorrhage usually causes minimal decreases in blood platelet counts. Platelet counts seldom decrease below  $100 \times 10^3/\mu\text{L}$  as a result of acute hemorrhage alone, although counts may decrease to as low as  $60 \times 10^3/\mu\text{L}$  in dogs with massive hemorrhage associated with anticoagulant rodenticide toxicity. Thrombocytopenia may be accentuated in animals with hemorrhage, if a large transfusion of packed erythrocytes or stored blood is given. These platelet-poor transfusions can have a dilutional effect on platelet numbers in blood. Platelet counts are often increased in animals with chronic hemorrhage as a result of increased platelet production.

## 7.9.2.6 Inherited Thrombocytopenia

Cavalier King Charles spaniels have a high incidence of thrombocytopenia ( $30\text{--}90 \times 10^3/\mu\text{L}$ ) with frequent macroplatelets present. This inherited disorder is transmitted as an autosomal recessive trait. Affected animals are asymptomatic, and platelet function appears to be normal.

## 7.9.3 Pseudothrombocytopenia

It is essential that a blood film estimation of platelet numbers be done as a quality control measure for each automated platelet count. The presence of platelet aggregates can result in erroneously low platelet counts. Platelet aggregates usually form when platelets are activated during blood sample collection and handling. EDTA-dependent pseudothrombocytopenia resulting from platelet aggregation has been reported in horses.

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When present, this aggregation can be prevented by the collection of blood samples with citrate, instead of EDTA, as the anticoagulant. Pseudothrombocytopenias may be reported in cats if whole-blood platelet counts are performed with electronic cell counters, because cat platelets are large and difficult to separate from erythrocytes on the basis of cell volume. The blood of some healthy Cavalier King Charles spaniels exhibits a population of macroplatelets that may not be counted as platelets by electronic cell counters, resulting in erroneously low platelet counts.

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## 7.9.4 Platelet Function Abnormalities

### 7.9.4.1 Acquired Platelet Function Defects

In addition to causing thrombocytopenia, antiplatelet antibodies may reduce platelet function. This may be explained by the finding that the GPIIb/IIIa fibrinogen receptor is apparently the most frequent target antigen in immune-mediated thrombocytopenia in humans. FDP released during fibrinolysis may reduce platelet

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function by antagonizing fibrinogen binding to GPIIb/IIIa. Disorders with high antibody concentrations, such as multiple myeloma, can cause coating of platelets and inhibit normal platelet function. Platelet defects resulting in reduced platelet function may occur in association with uremia and liver disease. Decreased platelet function can also occur after the administration of nonsteroidal antiinflammatory drugs, such as aspirin and phenylbutazone, which inhibit  $\text{TxA}_2$  synthesis. In addition, antihistamines, methylxanthines (theophylline and aminophylline), calcium channel blockers, halothane, barbiturates, bile acids, and certain antibiotics may interact with platelets and interfere with normal platelet aggregation. Platelet function may be reduced in various myeloproliferative disorders with abnormal platelet formation. Decreased platelet function has been reported in association with some infectious agents. This might be explained if platelets are activated in vivo and become hypofunctional when tested in vitro.

Enhanced platelet function may occur in disorders in which platelet activation occurs. Disorders in which platelets may be hyperactive include diabetes mellitus, nephrotic syndrome, erythropoietin treatment, neoplasia, feline infectious peritonitis virus infection, heartworm disease, and allergic respiratory disease.

7.9.4.2

## Inherited Platelet Function Defects

Inherited platelet defects (termed *thrombopathy* or *thrombopathia*) that result in excessive bleeding have been reported in animals. Animals with these disorders usually have normal platelet counts and normal platelet morphology. Deficiencies in the GPIIb/IIIa receptor account for platelet function defects in otterhounds and Great Pyrenees dogs. Both defects appear to be homologous to Glanzmann's thrombasthenia in humans and are referred to as *thromboasthenic thrombopathia*. Thrombopathies have been reported in thoroughbred and standardbred horses that had platelet function abnormalities consistent with thromboasthenic thrombopathia, but GPIIb/IIIa receptor assays were not done. Platelet-dense granule ( $\delta$ -granule) deficiency (platelet  $\delta$  storage pool disease) has been reported in pigs and American cocker spaniels. Platelet  $\delta$ -granule deficiency also occurs in Persian cats, Hereford cattle, and Aleutian mink as part of the inherited Chédiak-Higashi syndrome. Animals with Chédiak-Higashi syndrome have partial albinism with defects in granules of several tissues including skin, leukocytes, and platelets. The pathogenesis of the hereditary thrombopathies in foxhounds, Scottish terriers, boxers, mixed-breed dogs, basset hounds, and spitz dogs and in Simmental cattle are unclear, but the mechanisms appear to involve signal transduction or secretion defects. Persistent thrombopathies have been reported in two cats, but the specific nature of the defects was not determined. Platelets from gray collie dogs with cyclic hematopoiesis appear to have signal transduction and storage pool defects, but bleeding is not of clinical significance in this disorder.

7.9.4.3

## von Willebrand Disease

vWD is a heterogeneous inherited bleeding disorder resulting from quantitative and/or qualitative defects of vWF. It is by far the most common bleeding disorder in dogs, having been recognized in more than 50 breeds. vWD is classified into three general types based on vWF concentration in plasma, the multimeric structure of vWF, and clinical severity. Type 1 vWD has low vWF (less than 50% of normal) concentration in plasma, but the multimeric structure of vWF is normal. Type 1 vWD appears to be transmitted as an autosomal dominant trait with variable penetrance. Mildly affected dogs may exhibit no bleeding tendency. Most dogs with type 1 vWD that have bleeding tendencies have less than 20% of normal vWF concentration in plasma. Dogs with type 2 vWD have low plasma vWF concentrations, with a disproportionate loss of high-molecular-weight multimers. Dogs with type 3 vWD have virtually no vWF in plasma. In addition, factor VIII:C in plasma is generally moderately decreased. Dogs with type 2 and type 3 vWD have severe bleeding tendencies. Type 2 and type 3 vWD are transmitted as autosomal recessive traits.

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Type 1 vWD is common in several breeds including Doberman pinschers, German shepherds, golden retrievers, poodles, Pembroke Welsh corgis, and Shetland sheepdogs. Type 2 vWD has been reported in German shorthaired pointers and German wirehaired pointers. Type 3 vWD has been reported in several breeds including Chesapeake retrievers, Dutch Kooikers, Scottish terriers, and Shetland sheepdogs. vWD also occurs in pigs and has been reported in a Himalayan cat, a quarter horse, and a Simmental calf. Animals with vWD have prolonged bleeding times because vWF is needed for normal platelet adhesion to the subendothelium. Decreased vWF concentration may result in decreased factor VIII:C activity, because the binding of VIII:C to vWF prolongs the half-life of factor VIII:C in the circulation. However, the decrease in factor VIII:C is usually not sufficient to result in a significant prolongation of the APTT.

## 7.9.5 Thrombocytosis

Thrombocytosis refers to the presence of platelet counts above the reference interval. It generally occurs as a result of increased production of thrombopoietin or other factors such as IL-1, IL-3, IL-6, and IL-11. Secondary thrombocytosis may occur after or in association with hemorrhage. This is especially common when ongoing hemorrhage results in iron deficiency anemia. It may also occur with some hemolytic anemias, with various chronic inflammatory diseases, and as a rebound response to thrombocytopenia. Thrombocytosis occurs within 1 week after splenectomy, even though the same number of platelets is produced, because the splenic platelet storage capacity has been removed. The platelet count may return to normal several months after splenectomy.

Primary thrombocytosis occurs independently of the presence of thrombopoietin or other growth factors. It may be present in some myeloproliferative disorders. The term *thrombocythemia* is usually used to describe a myeloproliferative disorder that is characterized primarily by persistent, markedly elevated platelet counts. It may be viewed as the platelet counterpart of polycythemia vera. The diagnosis of thrombocythemia is made by ruling out other causes of high platelet counts.

## 7.10 COAGULATION DISORDERS

### 7.10.1 Acquired Coagulation Disorders

#### 7.10.1.1 Hypercoagulable State

The increased tendency for coagulation to occur without clinical signs or laboratory evidence of thrombosis is termed a *hypercoagulable state*. An increased risk of thrombosis may also be described as *thrombophilia* or a *prethrombotic state*. Potential causes include initial reactions that ultimately result in thrombosis or DIC (see discussion in the following sections), antithrombin III deficiency (e.g., nephrotic syndrome in dogs), and protein C deficiency (a hereditary disorder in horses). A hypercoagulable condition may develop in horses with severe colic as evidenced by decreased antithrombin III and protein C levels and increased thrombin-antithrombin complexes in plasma.

#### 7.10.1.2 Thrombosis

The pathogenesis of thrombosis may involve endothelial activation or injury; altered blood flow (turbulence or stasis); changes in coagulation factors, fibrinolytic factors, or their inhibitors; and platelet activation. Thrombosis may occur in association with immune-mediated, infectious, or traumatic vascular injury; neoplasia (especially hemangiosarcoma in dogs); autoimmune hemolytic anemia (common in dogs);

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immune-mediated thrombocytopenia; protein-losing nephropathy; glomerulonephropathies, hyperadrenocorticism; glucocorticoid therapy; acute pancreatic necrosis (in dogs); thrombocytosis; hypovolemia; heart disease (vegetative endocarditis, dirofilariasis, cardiomyopathy); and use of indwelling intravenous catheters. When thrombus formation is localized, platelet counts, coagulation test results, and FDP values are often normal.

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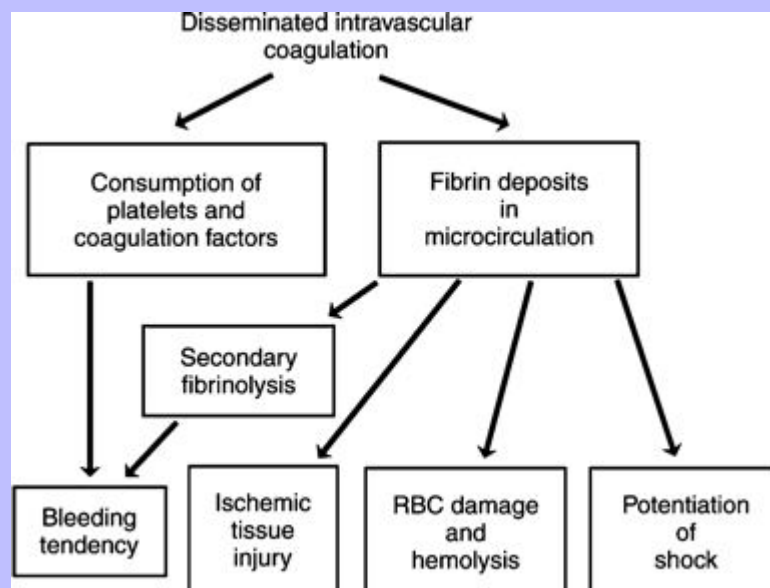
7.10.1.3

### Disseminated Intravascular Coagulation

DIC is a syndrome in which diffuse thrombosis and secondary fibrinolysis occur in small vessels. DIC is not a primary disorder. It always occurs in association with other clinical conditions. The formation of thrombi in vessels can result in tissue hypoxia and organ damage (Fig. 6-13). The consumption of coagulation factors and platelets in the formation of these thrombi creates a tendency for hemorrhage. This propensity to bleed is increased by subsequent fibrinolysis, which not only breaks down thrombi but produces FDPs that interfere with normal platelet aggregation and fibrin polymerization. DIC often occurs as a life-threatening event causing organ failure, hemorrhage, or both but may also occur in a chronic form without severe clinical signs. DIC can result in shock, and shock can potentiate DIC, resulting in a vicious cycle of events. Extensive local intravascular coagulation may occur in some dogs with hemangiosarcoma. Laboratory findings in these cases are similar to those seen in DIC, making differentiation of DIC from local intravascular coagulation difficult.

DIC may occur with disorders in which TF is exposed on cell surfaces, with disorders in which widespread vascular injury is present, with disorders resulting in widespread platelet activation, with disorders resulting in reduced blood flow, and in disorders with impaired removal of activated coagulation factors by the liver. Conditions that may induce DIC are shown in Box 6-1. Undoubtedly, other disorders can be added to this list.

Fig. 6-13



Pathophysiology of disseminated intravascular coagulation. RBC, Red blood cell.

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## 7.10.1.4 Liver Disease

The liver is the primary site for the synthesis of coagulation factors. Consequently, generalized liver disease may result in an increased bleeding tendency caused by decreased circulating coagulation factors. Because of these synthetic functions and the vascular nature of the liver, coagulation screening tests are generally done before liver biopsies are performed. Liver disorders may also contribute to the development of DIC.

## 7.10.1.5 Vitamin K Deficiency

Vitamin K is essential in a carboxylation reaction that results in the formation of active coagulation factors II, VII, IX, and X. Vitamin K deficiency may occur in malabsorptive syndromes (e.g., bile duct obstruction) or as a result of sterilization of the gut by prolonged use of antibiotics. Dicumarol, a product of moldy sweet clover, inhibits the vitamin K–dependent carboxylation reaction. Consequently, dicumarol toxicity can result in hemorrhage in cattle and other species consuming moldy clover. This discovery led to the development of related compounds that are now used as rodenticides and therapeutic anticoagulants. In animals that consume these anticoagulant rodenticides life-threatening bleeding disorders, characterized by markedly prolonged PT and APTT, develop. The PT test is generally reported to be more sensitive than the APTT test in the recognition of early anticoagulant rodenticide toxicity because of the short half-life of factor VII in the circulation, but the APTT was more often prolonged than the PT in horses with experimental brodifacoum toxicity.

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## 7.10.1.6 Snake Venoms

Snake venoms contain various components that have profound stimulatory or inhibitory effects on hemostatic mechanisms, including coagulation, fibrinolysis, platelet function, and vascular integrity. Snake venoms vary in fibrinolysin and procoagulant content. Most procoagulants exert their effect late in the clotting cascade by activating factor X or prothrombin, or by directly converting fibrinogen to fibrin. The venom from the Eastern diamondback rattlesnake contains the enzyme crotalase, which degrades fibrinogen. The Western diamondback rattlesnake venom either directly or indirectly activates plasminogen to plasmin, which subsequently results in fibrinogen proteolysis. Envenomation from both species results in blood that does not clot after collection. Platelet counts may be normal but are often decreased. Hemorrhage after rattlesnake bites is also potentiated by the presence of agents in venom that directly cause endothelial injury. Hemostatic abnormalities are not clinically significant features of water moccasin, copperhead, or coral snake envenomations.

### 7.10.1.6.1 BOX 6-1 Conditions That May Result in Disseminated Intravascular Coagulation

- Septicemia (various gram-negative and gram-positive bacteria)
- Viremia (infectious canine hepatitis, canine herpes, canine distemper, feline parvovirus, feline infectious peritonitis, African swine fever, blue tongue, and hog cholera)
- Protozoal parasites (babesiosis, trypanosomiasis, sarcocystosis, leishmaniasis, and cytauxzoonosis)
- Metazoal parasites (heartworms and lungworms)
- Marked tissue injury (heatstroke, trauma, and surgical procedures)

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- Intravascular hemolysis
- Obstetric complications
- Malignancy (hemangiosarcoma and disseminated carcinomas)
- Traumatic shock
- Liver disease
- Pancreatitis
- Gastric dilatation-volvulus
- Toxins (snake and insect venoms, aflatoxin, and insecticides)

## 7.10.2 Hereditary Coagulation Disorders

An inherited coagulation factor deficiency is considered when unexplained hemorrhage occurs or hemorrhage is protracted after surgery. Hereditary coagulation defects appear to be much more common in dogs than in other domestic animal species. The likelihood that a coagulation defect will result in clinically significant hemorrhage varies with the nature of the defect.

### 7.10.2.1 Intrinsic Coagulation Pathway Defects

Animals with factor XII deficiency have prolonged APTT and ACT, but they do not have a bleeding disorder. Consequently, it is generally accepted that factor XII is not involved in normal hemostasis. Like animals with factor XII deficiency, animals with prekallikrein deficiency generally do not exhibit an increased bleeding tendency, although excessive hemorrhage has been reported after castration of a Belgian horse with this deficiency. Factor XI deficiency has resulted in protracted bleeding after surgery in affected dogs and cattle.

Factor IX (hemophilia B) and factor VIII:C (hemophilia A, classical hemophilia) deficiencies are transmitted as X chromosome-linked recessive traits; consequently, these disorders are usually recognized in male animals. These deficiencies generally result in severe bleeding disorders.

### 7.10.2.2 Extrinsic Coagulation Pathway Defect

Factor VII deficiency has been reported in several breeds of dogs and occurs frequently in beagles. This deficiency results in mild disease with no overt bleeding tendency, except for increased bruising.

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### 7.10.2.3 Common Coagulation Pathway Defects

Factor X deficiency results in severe bleeding episodes in dogs. In American cocker spaniels, this deficiency usually results in stillborn pups or fatal bleeding episodes in the neonatal period. Prothrombin (factor II) deficiency has been reported in dogs with mild bleeding tendencies. Fibrinogen (factor I) deficiency causes mild to severe bleeding episodes in affected dogs and goats. Factor V and factor XIII deficiencies have not been reported in animals.

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## 7.10.2.4 Vitamin K–Dependent Coagulopathy

Vitamin K–dependent coagulopathies have been recognized in Devon rex cats and Rambouillet sheep. Affected animals are deficient in the enzyme  $\gamma$ -glutamyl carboxylase, resulting in reductions in the activities of factors II, VII, IX, and X. Some animals exhibit minimal bleeding tendencies, but fatal hemorrhagic episodes have occurred in affected cats. Periparturient hemorrhage and death generally occur in lambs with this disorder.

## 7.11 INTERPRETATION OF HEMOSTATIC TEST PROFILES

The use of a number of hemostatic tests concomitantly helps differentiate causes of hemorrhage, including simple thrombocytopenia; DIC; vWD; vasculitis; rodenticide toxicity; liver disease; and inherited defects of the intrinsic, extrinsic, and common coagulation pathways. Examples of hemostatic test profiles and their interpretations are given in the following list.

1. *Thrombocytopenia with normal APTT, PT, and FDP test results.* Thrombocytopenias without abnormalities in coagulation test results generally result from lack of production or enhanced platelet destruction. Bone marrow biopsies are needed to determine whether there is an abnormality in platelet production by megakaryocytes. Generalized marrow hypoplasia and aplasia are more common than pure megakaryocytic thrombocytopenias, which are rare in animals. Myelophthisis resulting from marrow neoplasia can also result in thrombocytopenia. Enhanced platelet destruction is the most common cause of thrombocytopenia, often occurring as a result of a primary or secondary immune-mediated process.
2. *Thrombocytopenia with prolonged APTT and PT and a positive FDP test result.* This profile suggests a consumption of both platelets and coagulation factors as occurs in DIC. The plasma antithrombin III concentration is generally low when measured. All five abnormalities listed will not be present in every animal with DIC. Of these measurements, the PT is most likely to be normal.
3. *Normal platelet count, prolonged APTT and PT, and a negative FDP test result.* This profile suggests either multiple coagulation defects, as can occur in rodenticide toxicity and liver disease, or less likely, an inherited defect in the common pathway. Platelet counts may be decreased in animals as a result of marked blood loss associated with rodenticide toxicity. Inexplicably, serum FDP concentrations have been reported to be increased in some dogs with anticoagulant rodenticide toxicity. Collection of blood from a heparinized intravenous line should also be considered as a factitious cause of this hemostatic profile.
4. *Normal platelet count and PT, prolonged APTT, and a negative FDP test result.* This profile suggests an inherited defect in the intrinsic pathway, inappropriately low plasma to anticoagulant ratio, or hemoconcentration.
5. *Normal platelet count and APTT, prolonged PT, and a negative FDP test result.* This profile suggests an inherited defect in the extrinsic pathway (e.g., factor VII deficiency) or early anticoagulant rodenticide toxicity in some species.
6. *Normal platelet count, APTT, and PT with a negative FDP test result in the presence of a bleeding diathesis.* This profile suggests a platelet function defect or vascular injury. A concomitant prolonged bleeding time suggests that a platelet function abnormality is present. Inherited platelet abnormalities or vWD could be present. vWD is diagnosed by measuring vWF in plasma by specialized laboratories. Some

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cases with severe vWF deficiency exhibit slightly prolonged APTT as a result of decreased factor VIII:C in plasma.

## 7.12 REFERENCES

- K Abrams, MY Yunusov, S Slichter, et al.: Recombinant human macrophage colony-stimulating factor-induced thrombocytopenia in dogs. *Br J Haematol.* **121**, 2003, 614–622.
- SR Bailey, MJ Andrews, J Elliott, et al.: Actions and interactions of ADP, 5-HT, histamine and PAF on equine platelets. *Res Vet Sci.* **68**, 2000, 175–180.
- DC Baker, SL Robbe, L Jacobson, et al.: Hereditary deficiency of vitamin-K-dependent coagulation factors in Rambouillet sheep. *Blood Coagul Fibrinolysis.* **10**, 1999, 75–80.
- MH Barton, DD Morris, N Norton, et al.: Hemostatic and fibrinolytic indices in neonatal foals with presumed septicemia. *J Vet Intern Med.* **12**, 1998, 26–35.
- SW Bateman, KA Mathews, Abrams-Ogg, ACG, et al.: Diagnosis of disseminated intravascular coagulation in dogs admitted to an intensive care unit. *J Am Vet Med Assoc.* **215**, 1999, 798–804.
- JD Bay, MA Scott, JE Hans: Reference values for activated coagulation time in cats. *Am J Vet Res.* **61**, 2000, 750–753.
- HJ Boermans, I Johnstone, WD Black, et al.: Clinical signs, laboratory changes and toxicokinetics of brodifacoum in the horse. *Can J Vet Res.* **55**, 1991, 21–27.
- AM Boisvert, CL Swenson, CJ Haines: Serum and plasma latex agglutination tests for detection of fibrin(ogen) degradation products in clinically ill dogs. *Vet Clin Pathol.* **30**, 2001, 133–140.
- A Boswood, CR Lamb, RN White: Aortic and iliac thrombosis in six dogs. *J Small Anim Pract.* **41**, 2000, 109–114.
- MK Boudreaux: Acquired platelet dysfunction. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 496–500.
- MK Boudreaux, S Ebbe: Comparison of platelet number, mean platelet volume and platelet mass in five mammalian species. *Comp Haematol Int.* **8**, 1998, 16–20.
- MK Boudreaux, DL Lipscomb: Clinical, biochemical, and molecular aspects of Glanzmann's thrombasthenia in humans and dogs. *Vet Pathol.* **38**, 2001, 249–260.
- E Brandt, A Ludwig, F Petersen, et al.: Platelet-derived CXC chemokines: Old players in new games. *Immunol Rev.* **177**, 2000, 204–216.
- P Brianceau, TJ Divers: Acute thrombosis of limb arteries in horses with sepsis: Five cases (1988-1998). *Equine Vet J.* **33**, 2001, 105–109.
- M Brooks: von Willebrand disease. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 509–515.
- M Caldin, T Furlanello, G Lubas: Validation of an immunoturbidimetric D-dimer assay in canine citrated plasma. *Vet Clin Pathol.* **29**, 2000, 51–54.
- MB Callan, ME Griot-Wenk, SG Hackner, et al.: Persistent thrombopathy causing bleeding in 2 domestic shorthaired cats. *J Vet Intern Med.* **14**, 2000, 217–220.
- MB Callan, R Walton, PF Jezyk, et al.: Thrombopathies causing bleeding in a boxer and mixed-breed dog. *J Am Anim Hosp Assoc.* **37**, 2001, 244–250.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- SA Center, K Warner, J Corbett, et al.: Proteins invoked by vitamin K absence and clotting times in clinically ill cats. *J Vet Intern Med.* **14**, 2000, 292–297.
- BH Chong: Heparin-induced thrombocytopenia. *J Thromb Haemost.* **1**, 2003, 1471–1478.
- BJ Darien: Acquired coagulopathy V: Thrombosis. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 574–580.
- BJ Darien: Fibrinolytic system. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 544–548.
- WJ Dodds: Other hereditary coagulopathies. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 1030–1036.
- BA Dolente, PA Wilkins, RC Boston: Clinicopathologic evidence of disseminated intravascular coagulation in horses with acute colitis. *J Am Vet Med Assoc.* **220**, 2002, 1034–1038.
- PA Gentry: Factor XI deficiency. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 1037–1041.
- PA Gentry: Platelet biology. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 459–466.
- PA Gentry, K Nyarko: Platelet lipids and prostaglandins. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 453–458.
- U Giger: Differing opinions on value of PIVKA test (letters to the editor). *J Am Vet Med Assoc.* **222**, 2003, 1070.
- CB Grindem, EB Breitschwerdt, PC Perkins, et al.: Platelet-associated immunoglobulin (antiplatelet antibody) in canine Rocky Mountain spotted fever and ehrlichiosis. *J Am Anim Hosp Assoc.* **35**, 1999, 56–61.
- TB Hackett, WE Wingfield, EM Mazzaferro, et al.: Clinical findings associated with prairie rattlesnake bites in dogs: 100 cases (1989-1998). *J Am Vet Med Assoc.* **220**, 2002, 1675–1680.
- FR Haselton, JS Alexander: Platelets and a platelet-released factor enhance endothelial barrier. *Am J Physiol.* **263**, 1992, L670–L678.
- RA Hutton, DA Warrell: Action of snake venom components on the haemostatic system. *Blood Rev.* **7**, 1993, 176–189.
- RC Jacoby, JT Owings, T Ortega, et al.: Biochemical basis for the hypercoagulable state seen in Cushing syndrome. *Arch Surg.* **136**, 2001, 1003–1006.
- JS Johnson, DC Baker, RC Bowen: Defective carboxylation of vitamin K dependent clotting factors in Rambouillet sheep (abstract). *Vet Pathol.* **38**, 2001, 570.
- IB Johnstone, L Viel, S Crane, et al.: Hemostatic studies in racing standardbred horses with exercise-induced pulmonary hemorrhage. Hemostatic parameters at rest and after moderate exercise. *Can J Vet Res.* **55**, 1991, 101–106.
- LA Jutkowitz, EA Rozanski, JA Moreau, et al.: Massive transfusion in dogs: 15 cases (1997-2001). *J Am Vet Med Assoc.* **220**, 2002, 1664–1669.
- JK Kingston, WM Bayly, DC Sellon, et al.: Effects of sodium citrate, low molecular weight heparin, and prostaglandin E1 on aggregation, fibrinogen binding, and enumeration of equine platelets. *Am J Vet Res.* **62**, 2001, 547–554.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- R Kirby, E Rudloff: Acquired coagulopathy VI: Disseminated intravascular coagulation. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 581–587.
- SL Kopplitz, MA Scott, LA Cohn: Effects of platelet clumping on platelet concentrations measured by use of impedance or buffy coat analysis in dogs. *J Am Vet Med Assoc*. **219**, 2001, 1552–1556.
- GR Lisciandro, M Brooks, JL Catalfamo: Contact factor deficiency in a German shorthaired pointer without clinical evidence of coagulopathy. *J Vet Intern Med*. **14**, 2000, 308–310.
- SC Lisciandro, A Hohenhaus, M Brooks: Coagulation abnormalities in 22 cats with naturally occurring liver disease. *J Vet Intern Med*. **12**, 1998, 71–75.
- CP Mandell: Essential thrombocythemia and reactive thrombocytosis. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 501–508.
- P Mansell: Hemophilia A and B. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 1026–1029.
- SL Marks: The buccal mucosal bleeding time. *J Am Anim Hosp Assoc*. **36**, 2000, 289–290.
- RS McConnico, K Copedge, KL Bischoff: Brodifacoum toxicosis in two horses. *J Am Vet Med Assoc*. **211**, 1997, 882–886.
- ME Mount, BU Kim, PH Kass: Use of a test for proteins induced by vitamin K absence or antagonism in diagnosis of anticoagulant poisoning in dogs: 325 cases (1987-1997). *J Am Vet Med Assoc*. **222**, 2003, 194–198.
- EJ Norman, Barron, RCJ, AS Nash, et al.: Evaluation of citrate-based anticoagulant with platelet inhibitory activity for feline blood cell counts. *Vet Clin Pathol*. **30**, 2001, 124–132.
- EJ Norman, RC Barron, AS Nash, et al.: Prevalence of low automated platelet counts in cats: Comparison with prevalence of thrombocytopenia based on blood smear estimation. *Vet Clin Pathol*. **30**, 2001, 137–140.
- CR Norris, SM Griffey, VF Samii: Pulmonary thromboembolism in cats: 29 cases (1987-1997). *J Am Vet Med Assoc*. **215**, 1999, 1650–1654.
- Y Ojiri, K Noguchi, N Shiroma, et al.: Uneven changes in circulating blood cell counts with adrenergic stimulation to the canine spleen. *Clin Exp Pharmacol Physiol*. **29**, 2002, 53–59.
- EH Olsen, AS McCain, EP Merricks, et al.: Comparative response of plasma VWF in dogs to up-regulation of VWF mRNA by interleukin-11 versus Weibel-Palade body release by desmopressin (DDAVP). *Blood*. **102**, 2003, 436–441.
- CM Otto, TM Rieser, MB Brooks, et al.: Evidence of hypercoagulability in dogs with parvoviral enteritis. *J Am Vet Med Assoc*. **217**, 2000, 1500–1504.
- KG Palmer, LG King, TJ Van Winkle: Clinical manifestations and associated disease syndromes in dogs with cranial vena cava thrombosis: 17 cases (1989-1996). *J Am Vet Med Assoc*. **213**, 1998, 220–224.
- HD Pedersen, J Haggstrom, LH Olsen, et al.: Idiopathic asymptomatic thrombocytopenia in Cavalier King Charles spaniels is an autosomal recessive trait. *J Vet Intern Med*. **16**, 2002, 169–173.
- J Peng, P Friese, RF Wolf, et al.: Relative reactivity of platelets from thrombopoietin- and interleukin-6-treated dogs. *Blood*. **87**, 1996, 4158–4163.
- MR Prater: Acquired coagulopathy II: liver disease. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 560–564.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- KJ Rickards, MJ Andrews, TH Waterworth, et al.: Differential effects of phosphodiesterase inhibitors on platelet activating factor (PAF)- and adenosine diphosphate (ADP)-induced equine platelet aggregation. *J Vet Pharmacol Ther.* **26**, 2003, 277–282.
- J Riehl, M Okura, E Mignot, et al.: Inheritance of von Willebrand's disease in a colony of Doberman pinschers. *Am J Vet Res.* **61**, 2000, 115–120.
- HR Roberts, DM Monroe, M Hoffman: Molecular biology and biochemistry of the coagulation factors and pathways of hemostasis. In Beutler, E, Lichtman, MA, Coller, BS, et al. (Eds.): *Williams hematology*. 6th ed., 2001, McGraw-Hill, New York, 1409–1434.
- FJ Roncales, JM Sancho: Coagulation activators. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 532–537.
- KE Russell, CB Grindem: Secondary thrombocytopenia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 487–495.
- KE Russell, PC Perkins, CB Grindem, et al.: Flow cytometric method for detecting thiazole orange-positive (reticulated) platelets in thrombocytopenic horses. *Am J Vet Res.* **58**, 1997, 1092–1096.
- KE Russell, PC Perkins, MR Hoffman, et al.: Platelets from thrombocytopenic ponies acutely infected with equine infectious anemia virus are activated in vivo and hypofunctional. *Virology.* **259**, 1999, 7–19.
- I Sato, GA Anderson, BW Parry: An interobserver and intraobserver study of buccal mucosal bleeding time in greyhounds. *Res Vet Sci.* **68**, 2000, 41–45.
- MA Scott: Immune-mediated thrombocytopenia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 478–486.
- JC Scott-Moncrieff, NG Treadwell, SM McCullough, et al.: Hemostatic abnormalities in dogs with primary immune-mediated hemolytic anemia. *J Am Anim Hosp Assoc.* **37**, 2001, 220–227.
- SE Sheafor, CG Couto: Anticoagulant rodenticide toxicity in 21 dogs. *J Am Anim Hosp Assoc.* **35**, 1999, 38–46.
- Smith, R III, JS Thomas: Quantitation of reticulated platelets in healthy dogs and in nonthrombocytopenic dogs with clinical disease. *Vet Clin Pathol.* **31**, 2002, 26–32.
- JE Steiss, WG Brewer, Jr., E Welles, et al.: Hematologic and serum biochemical reference values in retired greyhounds. *Comp Contin Educ Pract Vet.* **22**, 2000, 243–248.
- T Stokol, MB Brooks, HN Erb, et al.: D-dimer concentrations in healthy dogs and dogs with disseminated intravascular coagulation. *Am J Vet Res.* **61**, 2000, 393–398.
- T Stokol, M Brooks, H Erb, et al.: Evaluation of kits for the detection of fibrin(ogen) degradation products in dogs. *J Vet Intern Med.* **13**, 1999, 478–484.
- F Tablin: Platelet structure and function. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 448–452.
- S Tasker, PJ Cripps, AJ Macklin: Estimation of platelet counts on feline blood smears. *Vet Clin Pathol.* **28**, 1999, 42–45.
- JS Thomas, RA Green: Clotting times and antithrombin III activity in cats with naturally developing diseases: 85 cases (1984–1994). *J Am Vet Med Assoc.* **213**, 1998, 1290–1295.
- R Tsuchiya, K Kyotani, MA Scott, et al.: Role of platelet activating factor in development of thrombocytopenia and neutropenia in dogs with endotoxemia. *Am J Vet Res.* **60**, 1999, 216–221.
- PH Walz, BA Steficek, JC Baker, et al.: Effect of experimentally induced type II bovine viral diarrhea virus infection on platelet function in calves. *Am J Vet Res.* **60**, 1999, 1396–1401.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- DJ Weiss, J Rashid: The sepsis-coagulant axis: A review. *J Vet Intern Med.* **12**, 1998, 317–324.
- DJ Weiss, E Townsend: Evaluation of reticulated platelets in dogs. *Comp Haematol Int.* **8**, 1998, 166–170.
- MJ Wilkerson, W Shuman: Alterations in normal canine platelets during storage in EDTA anticoagulated blood. *Vet Clin Pathol.* **30**, 2001, 107–113.
- MJ Wilkerson, W Shuman, S Swist, et al.: Platelet size, platelet surface-associated IgG, and reticulated platelets in dogs with immune-mediated thrombocytopenia. *Vet Clin Pathol.* **30**, 2001, 141–149.
- RF Wolf, J Peng, P Friese, et al.: Erythropoietin administration increases production and reactivity of platelets in dogs. *Thromb Haemost.* **78**, 1997, 1505–1509.
- D Zelmanovic, EJ Hetherington: Automated analysis of feline platelets in whole blood, including platelet count, mean platelet volume, and activation state. *Vet Clin Pathol.* **27**, 1998, 2–9.
- KL Zimmerman: Drug-induced thrombocytopenia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 472–477.

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## 8 Chapter 7 Immunohematology

### 8.1 IMMUNE SYSTEM

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The immune system is an integrated network composed of various cell types, numerous cytokines, and certain plasma proteins that work in synergy to eliminate infectious agents, parasites, and noxious antigens; consequently, defects in the immune response result in increased susceptibility to these foreign invaders. Inappropriate or exaggerated immune responses result in immune-mediated tissue injury. Because a thorough review of immunology is beyond the scope of this text, the reader is referred to current immunology textbooks for more detailed information.

#### 8.1.1 Specific Immunity

Lymphocytes are the immunocompetent cells that respond to specific antigens. The production and function of lymphocyte types are discussed in [Chapters 3](#) and [5](#), respectively. B lymphocytes are primarily responsible for immunoglobulin (antibody) production; however, immunoglobulin production also requires the participation of T lymphocytes, macrophages, and dendritic cells. In contrast to B lymphocytes, which produce immunoglobulins that are carried in the blood (humoral immunity) to the site of a foreign antigen, T lymphocytes can migrate to the site of a foreign antigen (cellular immunity). T lymphocytes are involved in immune regulation, cytotoxicity, delayed-type hypersensitivity, and graft-versus-host reactions. T-helper ( $CD4^+$ ,  $CD8^-$ ) lymphocytes promote humoral immune responses and T-cytotoxic ( $CD4^-$ ,  $CD8^+$ ) lymphocytes play pivotal roles in cell-mediated immunity directed at fungi, protozoan organisms, and neoplastic cells. Suppressor and regulatory T lymphocytes function to maintain a balance between activation of the immune system and prevention of autoimmunity. Memory T lymphocytes may be triggered to proliferate by a number of antigen-presenting cells, including B lymphocytes, macrophages, and dendritic cells. However, only antigen-presenting dendritic cells are believed to be capable of inducing primary responses to antigens by naive T lymphocytes.

#### 8.1.2 Nonspecific Immunity

Nonspecific immunity involves neutrophils, macrophages, mast cells, eosinophils, basophils, and natural killer (NK) cells along with the complement system. In addition to their roles in phagocytosis and the production of various inflammatory cytokines, macrophages play a role in the processing and presentation of antigens to T lymphocytes. The production and function of these various cell types are discussed in [Chapters 3](#) and [5](#), respectively. The mucosal surfaces and their secretions are another component of the nonspecific defense network.

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### 8.2 TESTS FOR IMMUNE-MEDIATED DISORDERS

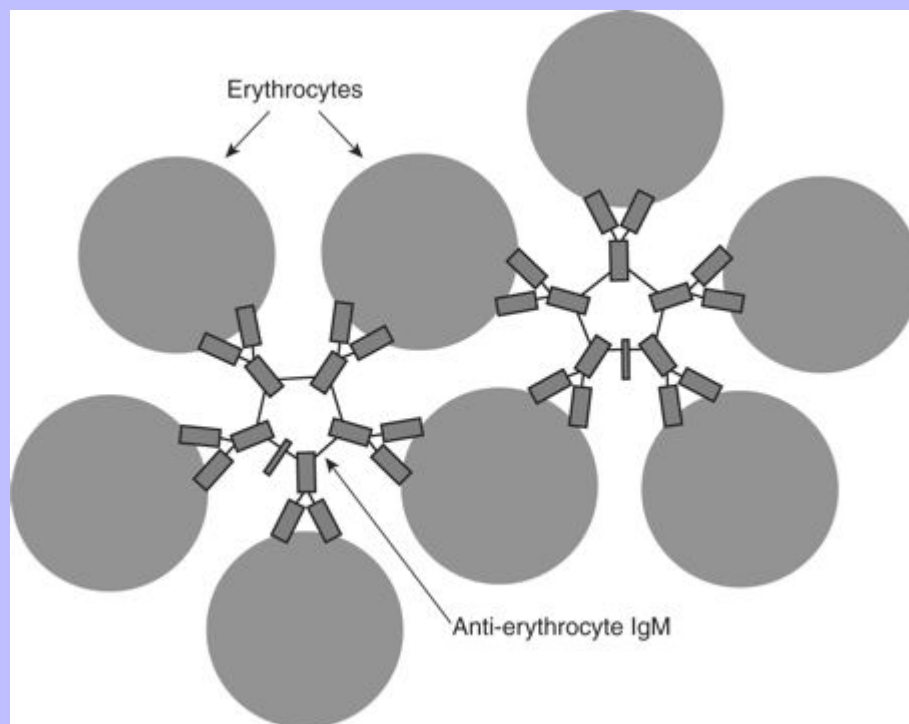
#### 8.2.1 Examination for Red Blood Cell Agglutination

When erythrocytes appear to clump in blood samples, it is important to differentiate autoagglutination (aggregation of erythrocytes together in clusters) from rouleau formation (adherence of erythrocytes together like a stack of coins). Rouleau formation is eliminated by washing erythrocytes in physiologic saline solution, but agglutination is not. This differentiation requires centrifugation of blood, removal of plasma, and

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resuspension of erythrocytes in saline solution. A rapid way to differentiate rouleaux from autoagglutination is to mix a drop of physiologic saline solution with a drop of anticoagulated blood on a glass slide and examine this as a wet mount with a microscope. This dilution reduces rouleau formation, but agglutination is not affected. The presence of autoagglutination indicates that the erythrocytes have increased surface-bound immunoglobulins. These immunoglobulins are usually of the IgM type, because of the presence of 10 antigen-binding sites per IgM molecule (Fig. 7-1). A direct antiglobulin test (DAT) is not needed if autoagglutination is present in saline solution—washed samples.

Fig. 7-1



Anti-erythrocyte IgM antibodies causing erythrocyte agglutination.

### 8.2.2 Tests for Anti-Erythrocyte Antibodies

Tests for anti-erythrocyte antibodies are done when autoagglutination is absent, but immune-mediated hemolytic anemia (IMHA) is still suspected.

#### 8.2.2.1 Direct Antiglobulin Test or Coombs' Test

In the DAT, washed erythrocytes from the patient and species-specific antisera against IgG, IgM, and the third component of complement (C3) are used to detect the presence of one or more of these factors on the surface of erythrocytes (Fig. 7-2). Unless clinical evidence of cold-agglutinin disease is present, this test is usually conducted only at 37°C, because a substantial number of healthy animals have positive test results when the test is run at low temperatures. In addition to autoimmune hemolytic anemia (AIHA), neonatal

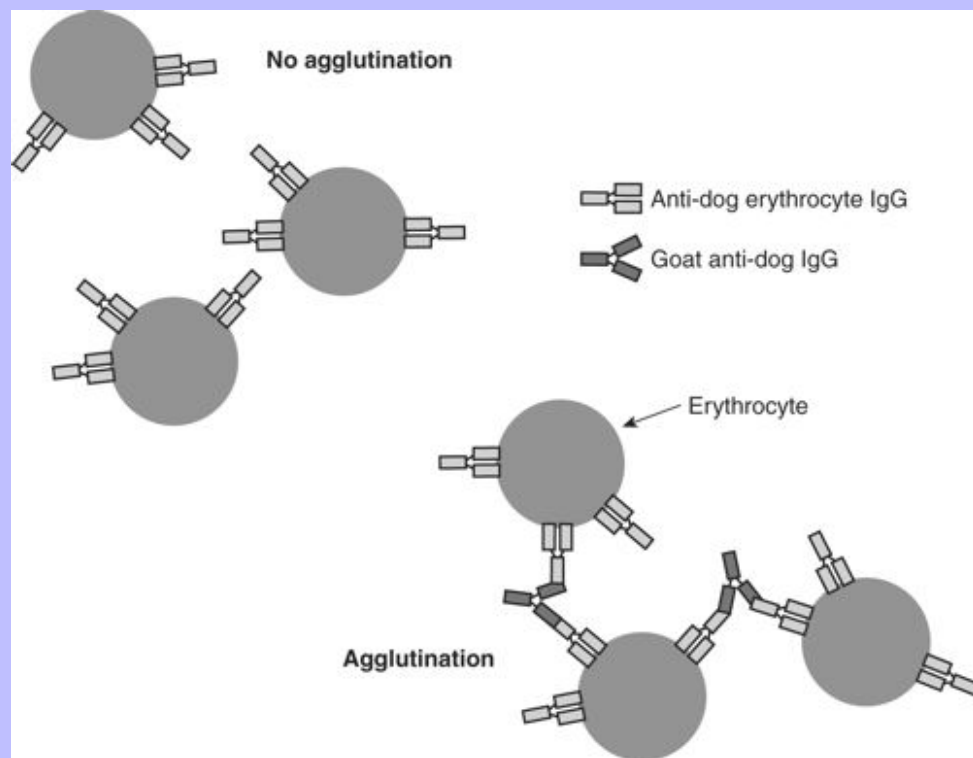
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isoerythrolysis (NI), and blood transfusion reactions, various infectious, parasitic, neoplastic, inflammatory, and other secondary immune-mediated diseases may be associated with a positive DAT result. If a drug-induced immune-mediated disorder is suspected, the offending drug should be included in the assay system.

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Fig. 7-2



Direct antiglobulin test (Coombs' test). The addition of anti-dog IgG antibody results in the agglutination of erythrocytes coated with dog IgG.

A negative DAT result does not rule out an IMHA. A false-negative test result may occur if there are insufficient quantities of antibody or complement on erythrocytes, if the ratio of antiglobulin in the reagent to antibody or complement on erythrocytes is not appropriate, if the test is performed with an incorrect species-specific reagent or performed at an improper temperature, if the animal has been treated with glucocorticoids for a week or more, or if the drug was not added to the test for an animal with a drug-induced IMHA.

### 8.2.2.2

#### Direct Immunofluorescence Flow Cytometry Assay

Fluorescein isothiocyanate-labeled antibodies against immunoglobulins of the species being evaluated are used to label erythrocyte-bound immunoglobulins, which are subsequently detected by flow cytometry. The direct immunofluorescence assay has greater sensitivity but lower specificity than the DAT assay for the evaluation of IMHA in dogs.

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## 8.2.2.3 Direct Enzyme-Linked Antiglobulin Test

The direct enzyme-linked antiglobulin test is an enzyme-linked immunosorbent assay (ELISA) that has been developed and evaluated for use in dogs. Regardless of the cause of the anemia, a majority of anemic dogs have increased erythrocyte-bound immunoglobulin, complement, or both when the direct enzyme-linked antiglobulin test is used. This test has high sensitivity but low specificity for the diagnosis of AIHA. It is also time-consuming and largely used as a research tool.

## 8.2.3 Blood Typing

Large numbers of protein and complex carbohydrate antigens occur on the external surfaces of erythrocytes. Some antigens are present on erythrocytes from all members of a species and others (called *alloantigens*) segregate genetically, appearing in some (but not all) members of a species. Erythrocyte alloantigens are detected serologically on the surfaces of erythrocytes by means of agglutination and hemolysis tests. On the basis of findings from detailed genetic studies, these erythrocyte alloantigens can be placed into blood groups. Blood groups have individual chromosomal loci, and each locus has from two to many allelic genes. Most blood groups derive their antigenicity from the carbohydrate composition of membrane-associated glycolipids and glycoproteins. Amino acid sequences in membrane proteins are the antigenic determinants in a lesser number of blood groups. Most blood group alloantigens are intrinsic components of erythrocyte membranes that are produced by the erythroid cells as they develop, but some alloantigens, such as dog erythrocyte antigen 7 (DEA 7) in dogs and the J system in cattle, are produced elsewhere in the body and released into plasma, where they attach to erythrocytes.

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Table 7-1 Frequency of blood type B in purebred cats in the United States

Type B Frequency	Breeds
25%-50%	Exotic Shorthair, British Shorthair, Cornish Rex, Devon Rex
5%-25%	Abyssinian, Birman, Persian, Himalayan, Somali, Sphynx, Scottish Fold, Japanese Bobtail
<5%	Main Coon Cat, Norwegian Forest Cat, domestic shorthair, domestic longhair
None	Siamese, Burmese, Tonkinese, Russian Blue, Oriental Shorthair, American Shorthair, Ocicat
Modified from Andrews (2000) and based on data published by Urs Giger and co-workers.	
Type A frequency is determined by subtracting type B frequency from 100% because type AB is extremely rare.	

Blood groups in domestic animals have been most extensively characterized in horses and cattle, in which blood typing has routinely been used for animal identification and parentage testing. Cattle have at least 11 blood groups with multiple alleles per group. On the basis of all the different combinations of factors that can occur, more than 2 trillion distinctly different blood-type profiles are possible in cattle. The use of blood groups for animal identification and parentage testing is being replaced by assays of DNA sequence.

Exposure to nonself alloantigens results in the production of alloantibodies. Erythrocyte alloantigens vary markedly in their potential to induce hemolytic anemia. Many erythrocyte alloantigens are weak (do not induce alloantibodies of high titer) or induce alloantibodies that do not act at normal body temperature. Fortunately, only a few erythrocyte alloantigens appear to be important in producing life-threatening hemolytic disease in animals. Erythrocyte alloantigens of clinical significance include DEA 1.1 (Aa<sub>1</sub>), and possibly DEA 1.2 (Aa<sub>2</sub>) in dogs, AB in cats, Aa and Qa in horses, and A and E in pigs.

Ideally, blood typing of donor and recipient animals for clinically significant erythrocyte alloantigens should be performed before all blood transfusions, as done in human medicine. This is generally not feasible in veterinary

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medicine because of the unavailability of in-house tests, cost considerations, or both. A practical alternative is to send out blood samples from potential donors for blood typing and select blood donors that are negative for clinically significant erythrocyte alloantigens. The use of blood from these donors coupled with cross-matching of donor and recipient samples will minimize the likelihood of severe transfusion reactions.

Blood typing of animals may be done before mating to identify animals with the same blood types and minimize the possibility of subsequent hemolytic reactions (NI) in newborn animals. This is most frequently done in mares that have previously given birth to foals that had NI after colostrum ingestion. It may also be considered in certain breeds of cats in which B-type blood is common ([Table 7-1](#)).

## 8.2.4 Blood Cross-Match Tests

Blood cross-match tests are used to detect the presence of hemagglutinating and hemolyzing antibodies in the serum of donor and recipient animals. Suspensions of washed erythrocytes are incubated with serum samples, centrifuged, and examined for the presence of hemolysis and gross and microscopic agglutination. The major cross-match is used to detect antibodies in the recipient's serum that are directed against the donor's erythrocytes. The minor cross-match is used to detect antibodies in the donor's serum that are directed against the recipient's erythrocytes. Autoagglutination or severe hemolysis in the patient's blood sample precludes the accurate performance of cross-match tests.

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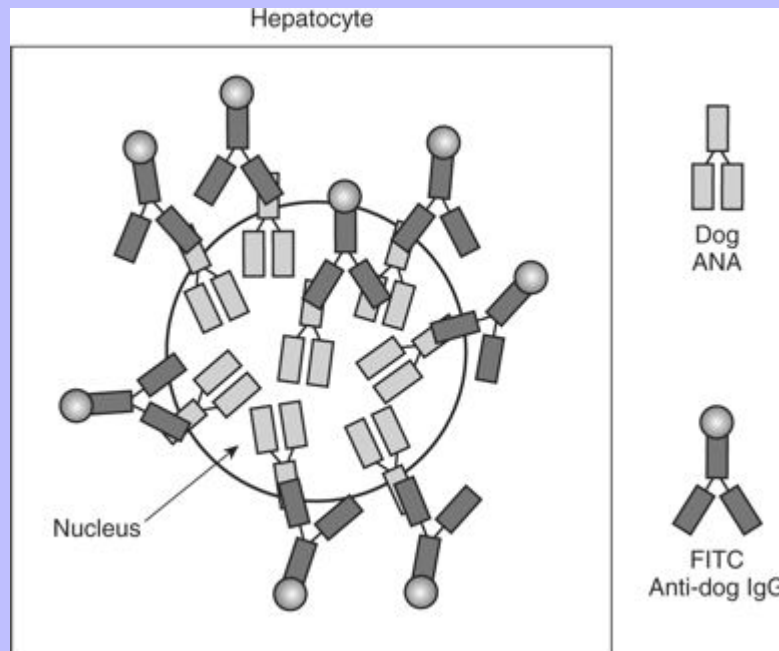
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The absence of agglutination or hemolysis in cross-match tests does not indicate that animals have similar blood types. It only indicates that preexisting antibodies were not detected and that an acute hemolytic transfusion reaction is highly unlikely. A delayed transfusion reaction can still occur if important alloantigen differences are present. The benefit of the transfusion is short-lived in delayed transfusion reactions because antibodies made against the donor's erythrocytes result in phagocytosis and removal of these erythrocytes within a few days.

## 8.2.5 Tests for Antinuclear Antibodies

The presence of circulating antinuclear antibodies (ANAs) is associated with various autoimmune diseases in humans and animals. ANAs are most often measured in dogs thought to have systemic lupus erythematosus (SLE). Studies indicate that ANAs in dogs are primarily of the IgG type. Canine ANAs are heterogenous and may be directed against various histone and nonhistone extractable nuclear antigen components of the nucleus, but not against native double-stranded DNA.

Fig. 7-3



Antinuclear antibody (ANA) test. Sections of liver are incubated with test serum, and after washing, the presence of ANA is demonstrated by using fluorescein isothiocyanate (FITC)-labeled antibodies against immunoglobulins of the species being tested.

### 8.2.5.1

#### ANA Test

An indirect immunofluorescent antibody technique is most widely used for ANA testing (Fig. 7-3). Typically, dilutions of a patient's serum are placed on a glass slide with tissue cells fixed to the surface. After time has been allowed for ANA present in the patient's serum to become bound to the nuclei, the slides are rinsed, and fluorescein-labeled antibodies directed against immunoglobulins of the same species as the patient are added. The slides are again rinsed, and the absence or presence of nuclear fluorescence (which occurs when ANAs are present) is determined by using a fluorescent microscope. Alternatively, an immunoperoxidase method may be used in place of the immunofluorescent method described. Frozen rodent liver sections have been used most frequently as the substrate in veterinary medicine, but a human epithelial cell line (HEp-2) appears to be a superior ANA substrate because of its low reactivity with normal serum and the ease with which the fluorescence pattern can be read. Titers greater than 1/25 and 1/100 are considered positive in dogs when HEp-2 and rat liver substrates are used, respectively.

ANA test results are usually positive in animals with SLE (97% to 100% in dogs with SLE); however, positive test results should be viewed with caution. Chronic bacterial infections (e.g., bacterial endocarditis), parasitism (e.g., heartworm disease), rickettsial infections, viral infections (e.g., feline leukemia virus [FeLV] and feline infectious peritonitis), and neoplasia can produce positive test results, although titers are usually low. Many healthy cats and up to 20% of healthy dogs are weakly ANA-positive.

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## 8.2.5.2 Lupus Erythematosus Cell Test

A lupus erythematosus (LE) cell is a leukocyte (usually a neutrophil) with a single, large reddish purple, amorphous inclusion that nearly fills the cytoplasm of the cell. This inclusion represents the nucleus of a damaged leukocyte that has been opsonized by ANA and complement and phagocytized by an intact leukocyte. LE cells occasionally form in vitro in stored anticoagulated blood, bone marrow, and joint fluids. The LE cell test is performed by promoting the formation of LE cells, which is done by rupturing leukocytes to expose their nuclear material either by forcing clotted blood through a sieve or mixing anticoagulated blood vigorously with glass beads. After the leukocytes have been ruptured, the samples are incubated to allow time for LE cell formation. Buffy coat smears are made, stained, and examined for the presence of LE cells. The presence of a single LE cell is considered a positive test result. With the ready availability of the ANA test, which is more sensitive and less labor-intensive to perform than the LE cell test, the latter test is now seldom done in veterinary laboratories. The advantages of the LE cell test are that it does not require species-specific reagents and is more specific for SLE than the ANA test.

## 8.2.6 Tests for Antiplatelet Antibodies

A number of tests have been developed to detect antiplatelet antibodies. These include a direct immunofluorescent test of bone marrow megakaryocytes and various means of detecting immunoglobulin bound to platelet surfaces. The microscopic detection of immunofluorescence of megakaryocytes is a subjective test that requires bone marrow aspiration for the purpose of obtaining megakaryocytes.

Increased platelet-bound immunoglobulins can be detected by flow cytometry, radioimmunoassay, ELISA, and microscopic platelet immunofluorescence techniques in animals; but these tests are not readily available. Most antiplatelet antibody in blood is bound to platelets; consequently, direct assays of the patient's platelets are more sensitive than indirect assays in which the patient's serum and platelets from a healthy control animal are used. Unfortunately, direct assays must be done within a few hours after blood sample collection. Platelets naturally have some immunoglobulin adsorbed to their surfaces. The amount of platelet-bound immunoglobulin can increase with time after sample collection; as a result, false-positive test results can be a significant problem with these assays. In an attempt to overcome this problem, different reference cutoff values have been proposed for assays in which fresh blood is used compared with assays in which day-old blood is used. Positive test results may also occur when immune complexes are adsorbed to platelets. At this time, none of the tests for antiplatelet antibodies are as readily available and cost-effective as the DAT for anti-erythrocyte antibodies.

## 8.3 PRIMARY IMMUNE-MEDIATED DISORDERS

Some degree of immune-mediated cellular destruction occurs in many infectious, parasitic, neoplastic, inflammatory, and drug-induced diseases. Disorders presented in this section do not appear to be associated with other diseases but represent primary immune-mediated disorders.

### 8.3.1 Transfusion Reactions

Naturally occurring anti-erythrocyte alloantibodies are defined as alloantibodies that occur in plasma in the absence of prior exposure to blood from another individual of the same species. In most animal blood groups, alloantibody formation results from prior exposure to different erythrocyte antigens through transfusion, pregnancy, or vaccination with products containing blood group antigens. Fortunately, naturally occurring

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alloantibodies of clinical significance are seldom found in animals; consequently, severe hemolytic transfusion reactions to unmatched erythrocytes generally do not occur at the time of the first blood transfusion. However, exceptions do occur, as in the case of the AB group in cats: cats with B-positive blood have naturally occurring anti-A antibodies with a high hemolytic titer. Less than 5% of domestic shorthair and longhair cats in the United States are type B, but up to 50% of purebred cats in some breeds are type B (see [Table 7-1](#)). The transfusion of type A blood into a type B cat can result in a life-threatening intravascular hemolytic reaction the first time a transfusion is given. In contrast, cats with type A blood have weak anti-B antibodies in their blood. Type B blood transfusions given to type A cats do not result in severe intravascular hemolysis, but the transfusion is not efficacious because the transfused erythrocytes are phagocytized and removed within a few days.

Although dogs have at least 12 blood groups, only the DEA 1.1 alloantigen regularly generates hemolysins in high enough titer to cause significant hemolytic transfusion reactions when dogs with antibodies to this blood group are again transfused with DEA 1.1–positive blood. The DEA 1.2 blood type may not be responsible for life-threatening intravascular hemolytic reactions, but previously sensitized DEA 1.2–negative dogs exhibit phagocytosis and removal of transfused erythrocytes within 1 day after administration of DEA 1.2–positive erythrocytes.

8.3.2

## Neonatal Isoerythrolysis

Animals with NI are healthy at birth, but hemolytic anemia develops within a few hours to a few days after ingestion of colostrum. Aa and Qa alloantigens are responsible for most cases of NI in horses: in mares that are negative for these factors, antibodies against them develop, and these antibodies are transferred to their newborn foals through colostrum. If the foals have acquired one or more of these erythrocyte alloantigens from the sire, a hemolytic reaction can occur. The dams become sensitized to these foreign erythrocyte antigens from leakage of fetal erythrocytes through the placenta during pregnancy or from exposure to fetal erythrocytes of the same blood type during a previous parturition. Generally, the first foal born is unaffected, but subsequent foals carrying the same foreign antigen(s) will likely have hemolytic anemia. NI occurs more frequently in mule foals than in horse foals because of an erythrocyte antigen that is not found in horses but is found in some donkeys and mules.

NI has been reported in some calves born to cows previously vaccinated for anaplasmosis or given other bovine-origin vaccines containing erythrocyte membranes. The most important blood group isoantigens involved in this disorder in calves are not yet known. The mating of a DEA 1.1–negative bitch with a DEA 1.1–positive stud dog can result in neonatal hemolytic anemia in DEA 1.1–positive pups, if the bitch has been sensitized to the DEA 1.1 antigen by previous blood transfusion or prior pregnancy.

NI can occur in type A kittens born to primiparous type B queens, because all adult type B cats naturally have high anti-A antibody titers. NI appears to be an important cause of neonatal death (fading kitten syndrome) in purebred cats from breeds with high frequencies of type B blood (see [Table 7-1](#)). Clinical signs that may be present include hemoglobinuria, pale mucous membranes, icterus, lethargy, weakness, tachypnea, tachycardia, collapse, and death. Tail-tip necrosis may occur in surviving kittens as a result of cold-acting IgM antibodies or localized thrombus formation.

Blood typing of prospective breeding animals can be done to minimize the possibility of NI in offspring. The possibility of NI developing in offspring can be evaluated by cross-matching the sire's erythrocytes with the dam's serum during pregnancy. If the potential for NI is identified before parturition, colostrum can be withheld from the offspring until a cross-match test can be done between the erythrocytes of the offspring and the serum

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of the mother. If an incompatibility is present, the neonatal animal can be foster-fed for 2 days and can be allowed to nurse from the mother after antibodies can no longer be absorbed as a result of gut closure.

## 8.3.3 Autoimmune Hemolytic Anemia

IMHA may be primary (autoimmune) or may occur as a result of rickettsial, bacterial, protozoal, or hemotropic mycoplasmal infections; neoplasia (especially lymphomas); or toxin or drug exposure. Immunization with combination vaccines has been suggested as a trigger of IMHA in dogs, but a subsequent study was not able to verify this association. In an autoimmune response, antibodies are directed against self-antigens on erythrocytes. In secondary immune-mediated disorders, the immune response is directed against foreign antigens or altered self-antigens, with inadvertent erythrocyte injury. A diagnosis of IMHA is made if autoagglutination (persisting after washing of erythrocytes with saline solution) or a positive DAT result is present. The DAT result is positive in about 60% of dogs with IMHA. The presence of spherocytosis strongly suggests that an immune-mediated process is present; however, other causes of spherocytosis including exposure to venoms, zinc toxicity, transfusion of stored blood, and hereditary disorders must be ruled out. Spherocytes are accurately recognized only in dogs, because the degree of central pallor is naturally less in other domestic animals. A diagnosis of AIHA, also called *idiopathic IMHA*, is reached by ruling out other disorders known to have concomitant IMHA.

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AIHA is common in dogs, but not in other domestic animal species. Retrospective studies have indicated an increased incidence of AIHA in certain dog breeds including Old English sheepdogs, cocker spaniels, border collies, poodles, English springer spaniels, and Irish setters. Canine AIHA is primarily seen in middle-aged dogs, with spayed female dogs being overrepresented. About 80% of the dogs with AIHA have spherocytosis and about two thirds have absolute reticulocytosis. Autoagglutination is a common finding. A regenerative response to this hemolytic anemia may be lacking, if the onset of anemia is acute or if antibodies, complement, or both are directed against reticulocytes or bone marrow precursor cells. Bilirubinemia and bilirubinuria are usually present. Hemoglobinemia with hemoglobinuria occurs in less than 10% of cases. Most cases of AIHA in dogs are mediated by IgG antibodies. IgM antibodies, complement, or both are most likely involved if autoagglutination or intravascular hemolysis is present.

A neutrophilia (often with a prominent left shift) is typically present in dogs with AIHA. Thrombocytopenia also occurs in a majority of the dogs with this disorder. The thrombocytopenia often appears to result from increased platelet utilization. Many dogs with AIHA appear to be in a hypercoagulable state at the time of diagnosis, with disseminated intravascular coagulation and multiorgan venous thrombosis (especially pulmonary thrombosis) being common sequelae that may result in death. In some instances the concurrent thrombocytopenia also appears to be autoimmune in origin (Evans' syndrome). AIHA may also be part of SLE, a multisystemic autoimmune disease.

About two thirds of dogs with IMHA appear to have AIHA. In contrast, IMHA in noncanine species is usually a secondary, rather than a primary, disorder. FeLV and *Mycoplasma haemofelis* are most commonly associated with IMHA in cats. In horses, IMHA has been documented most often with equine infectious anemia virus and after the administration of drugs such as penicillin.

## 8.3.4 Autoimmune Thrombocytopenia

Immune-mediated thrombocytopenia (IMT) occurs when immunoglobulin (primarily IgG) is bound to the surface of platelets, resulting in the premature removal of platelets by macrophages. The presence of IMT is detected by measuring immunoglobulin bound to the patient's platelets (direct assays) or by measuring

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immunoglobulin in patient serum that is capable of binding to platelets collected from a healthy animal of the same species (indirect assay). Direct assays are more sensitive than indirect assays for detecting IMT (see the previous section on tests for platelet-bound immunoglobulins).

IMT may be primary (autoimmune), or it may occur as a result of rickettsial, bacterial, viral, or parasitic infections; neoplasia; rheumatoid arthritis; or drug administration. Additionally, neonatal alloimmune thrombocytopenia has been reported in newborn horses, mules, and pigs. In this disorder, thrombocytopenia develops in the neonate after ingestion of colostrum containing antibodies against the newborn's platelets.

A diagnosis of autoimmune thrombocytopenia, also called *idiopathic thrombocytopenic purpura*, is made after other potential causes of IMT have been ruled out. A presumptive diagnosis of autoimmune thrombocytopenia is often confirmed by a positive response to glucocorticoid therapy alone or in combination with administration of vincristine, azathioprine, cyclophosphamide, danazol or splenectomy. Autoimmune thrombocytopenia may occur in association with AIHA (Evans' syndrome) or may be a component of SLE.

Autoimmune thrombocytopenia is common in dogs, and it occurs twice as often in females as it does in males. It tends to occur in middle-aged dogs, with an increased incidence reported in cocker spaniels, miniature and toy poodles, Old English sheep dogs, and German shepherds. Many dogs are first seen with bleeding problems in the absence of other signs of illness, but some animals have lethargy and weakness attributable to anemia. Cutaneous and mucosal petechial and ecchymotic hemorrhages, epistaxis, hyphema, retinal hemorrhages, hematemesis, and melena are common types of hemorrhage observed. Splenomegaly, hepatomegaly, lymphadenopathy, and fever are uncommon in dogs with autoimmune thrombocytopenia. In response to the thrombocytopenia, megakaryocyte numbers are usually increased in the bone marrow, but rare cases of amegakaryocytic thrombocytopenia have been reported in dogs and a single case has been reported in a cat.

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## 8.3.5 Idiopathic Immune-Mediated Neutropenia

Idiopathic immune-mediated neutropenia appears to be more common in dogs and cats than previously recognized. Animals may present with fever and malaise, but they are often asymptomatic. In these cases, neutropenia is typically identified during a routine hematologic evaluation as part of an annual physical examination or before administration of anesthesia. Some animals may also exhibit other evidence of immune-mediated disease including nonseptic meningitis, nonerosive polyarthritis, and vasculitis. Neutropenia is typically severe ( $<500$  neutrophils/ $\mu\text{L}$ ) without toxic cytoplasm in asymptomatic animals. A moderate lymphocytosis and mild thrombocytopenia may occur in cats. Granulocytic hyperplasia with few mature neutrophils is most likely to be present in bone marrow, but granulocytic hypoplasia may occur when antigens on early neutrophil precursors are targeted.

A number of diagnostic tests (including flow cytometry, immunofluorescence leukoagglutination, and radioimmunoassay) have been developed to detect increased anti-neutrophil antibodies. Unfortunately, none of these tests have proven to be clinically reliable. Consequently, a diagnosis of immune-mediated neutropenia is generally made by excluding other causes of neutropenia. A substantial increase in neutrophil numbers in blood within 1 to 3 days after initiation of immunosuppressive treatments with corticosteroids provides retrospective evidence of an immune-mediated neutropenia.

## 8.3.6 Systemic Lupus Erythematosus

SLE is a chronic, autoimmune disease characterized by the production of a variety of autoantibodies (including ANAs) that result in immune-mediated injury of multiple organs. SLE is fairly common in dogs but is rare in

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cats and horses. Dogs with SLE have reduced CD8<sup>+</sup> T-lymphocyte numbers in blood, suggesting that loss of a subset of CD8<sup>+</sup> suppressor T lymphocytes may be responsible for the uncontrolled production of autoantibodies by B lymphocytes. Possible manifestations of SLE are persistent or recurring fever, nonerosive polyarthritis, renal disorders, facial or mucocutaneous dermatitis, lymphadenopathy and/or splenomegaly, leukopenia, anemia (often DAT-positive), thrombocytopenia, polymyositis, and pleuropericarditis. A diagnosis of SLE should be considered when several of these inflammatory processes are recognized in a patient. The presence of a positive ANA test or LE cell test result helps confirm the diagnosis (see sections on tests for ANAs). About 90% of dogs with SLE have ANA titers greater than 1/256; but few dogs with other diseases, and rarely, even apparently healthy control dogs (most notably German shepherds) may have high ANA titers.

## 8.4 TESTS FOR IMMUNE DEFICIENCY DISORDERS

### 8.4.1 Neutrophil Function Tests

Numerous steps are required for neutrophil chemotaxis, phagocytosis, and killing of bacteria; consequently, a variety of tests are needed to assess neutrophil function. These tests are not available in most commercial laboratories but are done in a limited number of research facilities. Chemotaxis assays measure the ability of neutrophils to migrate in the direction of various chemoattractants. The ability to phagocytize microbes can be determined microscopically. Microbial phagocytosis and killing can be evaluated by means of bacterial culture, after incubation of bacteria, serum, and neutrophils together. The nitroblue tetrazolium reduction test and the chemiluminescence test detect the presence of the oxidant burst needed for normal bacterial killing. These tests are screening tests; more specialized tests are required to demonstrate the specific nature of an inherited defect.

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### 8.4.2 Lymphocyte Assays

Most circulating lymphocytes are T lymphocytes. Consequently, the presence of a normal absolute blood lymphocyte count tends to rule out a generalized defect in T-lymphocyte production. Lymphocyte blastogenic assays are used to determine the responsiveness of lymphocytes to various mitogens, which variably stimulate different lymphocyte subsets. T-lymphocyte function may also be assessed in vitro by using leukocyte migration inhibition assays, cytokine release assays, and cytotoxicity assays. Subpopulations of lymphocytes can be quantified by using fluorescence labeling of surface molecules and flow cytometry. For example, with the use of flow cytometry, cats with feline immunodeficiency virus (FIV) infections have been shown to have reduced populations of CD4<sup>+</sup> lymphocytes.

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### 8.4.3 Serum Immunoglobulin Assays

A variety of methods may be used to determine whether immunoglobulin deficiencies are present. Routine serum protein electrophoresis may be used as a screening test, because immunoglobulins account for all of the protein that migrates in the  $\gamma$  region and some of the protein that migrates in the  $\beta$  region of electrophoretic gels. A low  $\gamma$ -globulin concentration indicates an immunoglobulin (usually IgG) deficiency. Various immunoelectrophoresis techniques can be used to make qualitative and quantitative serum immunoglobulin measurements. Specific immunoglobulin classes may be quantified by various methods, including single radial immunodiffusion, ELISA, rocket immunoelectrophoresis, and laser nephelometry. Reference intervals are generally established with serum from adult animals. Consequently, it is important not to overemphasize seemingly low serum immunoglobulin values in young animals. For example, in dogs, serum IgM

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concentrations do not increase to adult reference intervals until several months after birth; and serum IgG and IgA values may not reach adult reference values until the age of 1 year or older (in the case of IgA).

Several semiquantitative tests including zinc sulfate turbidity, glutaraldehyde coagulation, sodium sulfite precipitation, and immunoassays have been used to screen for the failure of the passive transfer of immunity through colostrum in neonatal animals. Enzyme immunoassays and turbidimetric tests are commercially available for the measurement of IgG in foals. A turbidimetric assay (Equine IgG Assay; Corporation for Advanced Application, Newburg, WI) is currently being used in our laboratory at the University of Florida.

## 8.5 IMMUNODEFICIENCY DISORDERS

### 8.5.1 Clinical Signs

Animals with immunodeficiencies generally have recurrent and chronic infections. Common conditions that may be present include respiratory tract infections, diarrhea, dermatitis, pyoderma, otitis, and growth retardation. Infections with opportunistic organisms, such as *Pneumocystis* and *Cryptosporidium* species, also suggest the presence of an immunodeficiency. Animals with B-lymphocyte defects generally have increased susceptibility to bacterial infections; and animals with T-lymphocyte defects typically have increased susceptibility to viral, fungal, and protozoal infections. Animals with neutrophil defects generally have cutaneous and systemic infections with pyogenic bacteria.

### 8.5.2 Inherited Neutrophil Defects

A number of inherited defects of neutrophils have been described. These include Chédiak-Higashi syndrome in several animal species,  $\beta_2$ -integrin adhesion molecule deficiencies in dogs and cattle, bactericidal defects in dogs, and cyclic hematopoiesis in gray collies. These disorders are discussed in [Chapter 5](#).

### 8.5.3 Severe Combined Immunodeficiency

The production of T and B lymphocytes is deficient in severe combined immunodeficiency (SCID) syndromes. SCID is transmitted as an autosomal recessive trait in Arabian foals. Affected foals have few or no circulating lymphocytes, hypoplasia of the primary and secondary lymphoid organs, and an inability to produce antibodies because of the absence of mature T and B lymphocytes. Failure to produce mature lymphocytes results from a mutation in the gene that encodes for the catalytic subunit of DNA-dependent protein kinase (DNA-PK). DNA-PK is required for the gene rearrangement process that provides the antigen receptors on T and B lymphocytes. The serum of foals collected before suckling normally contains some IgM, but this is not the case in foals with SCID. If affected foals suckle successfully shortly after birth, they acquire immunoglobulins from the mare and generally appear healthy. Once maternal immunoglobulins are catabolized, foals with SCID become susceptible to a variety of overwhelming infections by pathogens, such as *Pneumocystis carinii* and *Cryptosporidium parvum*, and they generally die by 4 to 6 months of age.

SCID has recently been reported in Jack Russell terriers. Like Arabian foals, affected pups also have a defect in the catalytic subunit of DNA-PK that appears to be transmitted as an autosomal recessive trait. Affected pups have profound lymphopenia, decreased serum immunoglobulin concentrations, and hypoplasia of all lymphoid organs. These dogs with SCID generally die between 8 and 14 weeks of age.

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An X-linked SCID syndrome has been recognized in basset hounds and Cardigan Welsh corgis. Affected male dogs fail to thrive, exhibit increased susceptibility to bacterial and viral pathogens, lack palpable peripheral lymph nodes, and generally die by 4 months of age unless they are housed in a germ-free environment. These dogs are unable to make IgG or IgA but have normal serum IgM concentrations. Absolute blood lymphocyte counts are decreased or in the low to normal range. B-lymphocyte percentages are generally normal, and T-lymphocyte percentages vary from absent to near normal. Blood lymphocytes are unresponsive to T-lymphocyte mitogens. Mutations of the common  $\gamma$ -chain gene that encodes for essential components of interleukin (IL)–2, IL-4, IL-7, IL-9, and IL-15 receptors have been shown to cause the X-linked SCID syndrome in dogs.

## 8.5.4 Serum Immunoglobulin Deficiencies

A heterogeneous group of disorders can result in reduced serum immunoglobulin concentrations in humans and animals. Common variable immunodeficiency in humans is characterized by reduced immunoglobulin (IgG, IgA, and/or IgM) concentrations in serum and increased susceptibility to infections. The number of B lymphocytes in blood is generally normal, but there appears to be a defect in the maturation of B lymphocytes to plasma cells in some humans. Other human patients may be deficient in helper T lymphocytes or have excessive numbers of cytotoxic T lymphocytes. Symptoms generally do not develop until young adulthood, but symptoms may be observed in children as young as 10 years.

Common variable immunodeficiency has been reported in a 12-year-old quarter horse with a persistent, multifocal bacterial infection and markedly reduced concentrations of IgG, IgG(T), IgM, and IgA in serum. The total lymphocyte count was normal; however, B lymphocytes were not detected in blood, and numbers of B lymphocytes were markedly reduced in lymph nodes and bone marrow. Total blood lymphocyte counts may be normal, because B lymphocytes generally comprise a low percentage of total blood lymphocytes. Other horses with marked deficiencies in one or more IgG types, which may represent variations of this disorder, have been described. Common variable immunodeficiency has been reported in seven young (<1 year old) miniature dachshunds with *Pneumocystis carinii* pneumonia. Abnormal findings included a lack of B lymphocytes in lymph nodes, decreased serum concentrations of all immunoglobulins measured, and abnormal responses of blood lymphocytes to mitogens. Similar laboratory findings were reported in a young Dogue de Bordeaux dog.

A late-onset immunodeficiency that is similar to common variable immunodeficiency in humans has been reported in shar-pei dogs. Increased susceptibility to infection, beginning at about 3 years of age, is reported. B-lymphocyte and T-lymphocyte abnormalities are present, and one or more serum immunoglobulin (IgA, IgM, or IgG) concentrations are low. Serum IgA and IgG deficiencies have also been reported in related Weimaraner and related rottweiler pups.

A possible X-linked agammaglobulinemia has been described in thoroughbred and standardbred foals that have no identifiable B lymphocytes. A number of selective immunoglobulin deficiencies have been described in domestic animals. These include IgM deficiency in horses and dogs, IgG deficiency in a foal, IgG<sub>2</sub> deficiency in cattle, and IgA deficiency in dogs.

Transient hypogammaglobulinemia may occur in young animals, if the onset of IgG and IgA production by the neonate is delayed after the disappearance of maternal antibodies at about 2 months of age. Deficient animals are susceptible to bacterial infections until their immune systems become fully functional at about 6 months of age.

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## 8.5.5 Complement Deficiency

An inherited deficiency of the third component of complement (C3) has been reported as an autosomal recessive trait in Brittany spaniels. Homozygous affected animals have recurrent sepsis, pneumonia, pyometra, and wound infections. Humoral immune response to both T-lymphocyte-dependent and T-lymphocyte-independent antigens is defective in affected dogs.

## 8.5.6 Viral Immune Deficiency Disorders

A number of viral immune deficiency disorders have been recognized in animals, a few of which are listed here. Feline acquired immunodeficiency syndrome is caused by FIV. Infected cats may be asymptomatic for months to years before signs of severe, chronic inflammatory diseases are observed. Increased susceptibility to infectious agents is associated with neutropenia, lymphopenia, or both and decreased numbers of CD4+ T lymphocytes. A similar syndrome has been reported in primates with simian immunodeficiency virus infections.

FeLV is a potent immunosuppressive virus. Persistently infected cats often exhibit neutropenia, lymphopenia, or both. Severe T-lymphocyte dysfunction is present, but B-lymphocyte function is generally only mildly impaired. FeLV-positive cats are predisposed to a variety of secondary infections. Other infectious diseases found to induce secondary immune deficiencies of T lymphocytes, B lymphocytes, or both include canine distemper virus, fetal equine herpes virus I infection, and bovine viral diarrhea virus in calves.

## 8.5.7 Failure of Passive Transfer of Immunoglobulins

Failure of passive transfer of immunoglobulins is an acquired immunodeficiency disorder. Before suckling, newborn domestic animals have extremely low amounts of immunoglobulin in their plasma. Colostrum is rich in IgG and IgA but also contains some IgM. Colostral immunoglobulins (especially IgG) can be absorbed intact through the small intestine of animals during the first day of life. If insufficient or poor-quality colostrum is produced, if the intake of colostrum is inadequate, or if there is a failure of intestinal absorption, the neonate may not obtain sufficient antibodies to provide the necessary protection against bacterial infections (especially septicemia). The reader is referred to the section on serum immunoglobulin assays for tests used to detect the failure of passive transfer. Reference values for plasma IgG in neonatal animals vary, depending on the species being analyzed and the method used. In general, serum IgG concentrations below 400 mg/dL are considered inadequate, concentrations from 400 to 800 mg/dL are considered marginal, and concentrations greater than 800 mg/dL are considered sufficient, when measured in foals 1 to 2 days after birth. Serum IgG concentration is considered adequate in calves if it exceeds 1000 mg/dL.

## 8.6 REFERENCES

- CB Andreasen, JA Roth: Neutrophil functional defects. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 356–365.
- GA Andrews: Red blood cell antigens and blood groups in the dog and cat. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 767–773.
- RN Barker: Anemia associated with immune responses. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 169–177.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- TG Bell, KL Butler, HB Sill, et al.: Autosomal recessive severe combined immunodeficiency of Jack Russell terriers. *J Vet Diagn Invest.* **14**, 2002, 194–204.
- AT Bowling: Red blood cell antigens and blood groups in the horse. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 774–777.
- J Bücheler: Fading kitten syndrome and neonatal isoerythrolysis. *Vet Clin North Am Small Anim Pract.* **29**, 1999, 853–870.
- V Buechner-Maxwell, MA Scott, L Godber, et al.: Neonatal alloimmune thrombocytopenia in a quarter horse foal. *J Vet Intern Med.* **11**, 1997, 304–308.
- CA Calvert, C Sammarco, C Pickus: Positive Coombs' test results in two dogs treated with amiodarone. *J Am Vet Med Assoc.* **216**, 2000, 1933–1936.
- L Chabanne, C Fournel, M Monestier, et al.: Canine systemic lupus erythematosus. Part I. Clinical and biologic aspects. *Comp Contin Educ Pract Vet.* **21**, 1999, 135–141.
- CG Couto: Immune-mediated neutropenia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 815–818.
- ME Dawes, JW Tyler, D Hostetler, et al.: Evaluation of a commercially available immunoassay for assessing adequacy of passive transfer in calves. *J Am Vet Med Assoc.* **220**, 2002, 791–793.
- MJ Day: Immune-mediated hemolytic anemia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 799–806.
- MJ Day: Possible immunodeficiency in related rottweiler dogs. *J Small Anim Pract.* **40**, 1999, 561–568.
- MJ Day: Systemic lupus erythematosus. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 819–826.
- MJ Day, C Power, J Oleshko, et al.: Low serum immunoglobulin concentrations in related Weimaraner dogs. *J Small Anim Pract.* **38**, 1997, 311–315.
- Q Ding, L Bramble, V Yuzbasiyan-Gurkan, et al.: DNA-PKcs mutations in dogs and horses: Allele frequency and association with neoplasia. *Gene.* **283**, 2002, 263–269.
- LR Etzel, RE Strohhahn, JK McVicker: Development of an automated turbidimetric immunoassay for quantification of bovine serum immunoglobulin G. *Am J Vet Res.* **58**, 1997, 1201–1205.
- PJ Felsburg: IgA deficiency. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 992–994.
- PJ Felsburg: Immunodeficiency disease. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 260–265.
- MJ Flaminio, V LaCombe, CW Kohn, et al.: Common variable immunodeficiency in a horse. *J Am Vet Med Assoc.* **221**, 2002, 1296–1302, 1267.
- CL Garon, MA Scott, KA Selting, et al.: Idiopathic thrombocytopenic purpura in a cat. *J Am Anim Hosp Assoc.* **35**, 1999, 464–470.
- U Giger, J Bücheler, DF Patterson: Frequency and inheritance of A and B blood types in feline breeds of the United States. *J Hered.* **82**, 1991, 15–20.
- U Giger, M Griot-Wenk, J Bücheler, et al.: Geographical variation of the feline blood type frequencies in the United States. *Feline Pract.* **19**, 1991, 21–27.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- CB Grindem, EB Breitschwerdt, PC Perkins, et al.: Platelet-associated immunoglobulin (antiplatelet antibody) in canine Rocky Mountain spotted fever and ehrlichiosis. *J Am Anim Hosp Assoc.* **35**, 1999, 56–61.
- ME Griot-Wenk, U Giger: Feline transfusion medicine — Blood types and their clinical importance. *Vet Clin North Am Small Anim Pract.* **25**, 1995, 1305–1322.
- PG Hoyt, MS Gill, KL Angel, et al.: Corticosteroid-responsive thrombocytopenia in two beef cows. *J Am Vet Med Assoc.* **217**, 2000, 717–720.
- DC Lewis: Immune-mediated thrombocytopenia. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 807–814.
- R Lobetti: Common variable immunodeficiency in miniature dachshunds affected with *Pneumocystis carinii* pneumonia. *J Vet Diagn Invest.* **12**, 2000, 39–45.
- JM MacLeay: Neonatal isoerythrolysis involving the Qc and Db antigens in a foal. *J Am Vet Med Assoc.* **219**, 2001, 79–81, 50.
- PM McManus, LE Craig: Correlation between leukocytosis and necropsy findings in dogs with immune-mediated hemolytic anemia: 34 cases (1994-1999). *J Am Vet Med Assoc.* **218**, 2001, 1308–1313.
- PM McManus, C Litwin, L Barber: Immune-mediated neutropenia in 2 dogs. *J Vet Intern Med.* **13**, 1999, 372–374.
- K Meek, L Kienker, C Dallas, et al.: SCID in Jack Russell terriers: A new animal model of DNA-PKcs deficiency. *J Immunol.* **167**, 2001, 2142–2150.
- A Niggemeier, HF Haberstroh, VE Nelson, et al.: An accidental transfusion of a type A kitten with type B blood causes a transient switch from blood type A to B. *J Vet Intern Med.* **14**, 2000, 214–216.
- SJ Noble, PJ Armstrong: Bee sting envenomation resulting in secondary immune-mediated hemolytic anemia in two dogs. *J Am Vet Med Assoc.* **214**, 1999, 1026–1027.
- Penedo, MCT: Red blood cell antigens and blood groups in the cow, pig, sheep, goat, and llama. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 778–782.
- LE Perryman: Severe combined immunodeficiency in Arabian foals. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 988–991.
- N Pusterla, JB Pusterla, SJ Spier, et al.: Evaluation of the SNAP foal IgG test for the semiquantitative measurement of immunoglobulin G in foals. *Vet Rec.* **151**, 2002, 258–260.
- KA Quigley, BJ Chelack, DM Haines, et al.: Application of a direct flow cytometric erythrocyte immunofluorescence assay in dogs with immune-mediated hemolytic anemia and comparison to the direct antiglobulin test. *J Vet Diagn Invest.* **13**, 2001, 297–300.
- S Ramirez, SD Gaunt, JJ McClure, et al.: Detection and effects on platelet function of anti-platelet antibody in mule foals with experimentally induced neonatal alloimmune thrombocytopenia. *J Vet Intern Med.* **13**, 1999, 534–539.
- ME Reimer, GC Troy, LD Warnick: Immune-mediated hemolytic anemia: 70 cases (1988-1996). *J Am Anim Hosp Assoc.* **35**, 1999, 384–391.
- MA Scott: Granulocyte and platelet antigens. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 783–788.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- MA Scott, L Kaiser, JM Davis, et al.: Development of a sensitive immunoradiometric assay for the detection of platelet surface-associated immunoglobulins in thrombocytopenic dogs. *Am J Vet Res.* **63**, 2002, 124–129.
- JC Scott-Moncrieff, NG Treadwell, SM McCullough, et al.: Hemostatic abnormalities in dogs with primary immune-mediated hemolytic anemia. *J Am Anim Hosp Assoc.* **37**, 2001, 220–227.
- T Stokol, JT Blue, TW French: Idiopathic pure red cell aplasia and nonregenerative immune-mediated anemia in dogs: 43 cases (1988-1999). *J Am Vet Med Assoc.* **216**, 2000, 1429–1436.
- S Tasker, AJ Mackin, MJ Day: Primary immune-mediated thrombocytopenia in a cat. *J Small Anim Pract.* **40**, 1999, 127–131.
- IR Tizard: In *Veterinary immunology. An introduction*. 6th ed., 2000, WB Saunders, Philadelphia.
- KJ Wardrop: Clinical blood typing and crossmatching. In Feldman, BF, Zinkl, JG, Jain, NC (Eds.): *Schalm's veterinary hematology*. 5th ed., 2000, Lippincott Williams & Wilkins, Philadelphia, 795–798.
- JL Whiting, JB David: Neonatal isoerythrolysis. *Comp Contin Educ Pract Vet.* **22**, 2000, 968–976.
- MJ Wilkerson, E Davis, W Shuman, et al.: Isotype-specific antibodies in horses and dogs with immune-mediated hemolytic anemia. *J Vet Intern Med.* **14**, 2000, 190–196.

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## 9 Chapter 8 Clinical Chemistry

*A physician who depends on the laboratory to make his diagnosis is probably inexperienced; one who says that he does not need a laboratory is uninformed. In either instance the patient is in danger.*

J. A. Halsteda

### 9.1 DIAGNOSTIC ENZYMOLOGY

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Diagnostic enzymology is the area of laboratory medicine that is involved in the study and application of enzyme activity as an aid for the recognition or diagnosis of disease, as a monitor of disease activity, and in the evaluation of the response to treatment. Enzymes are protein catalysts that accelerate biochemical reactions within cells. They undergo a physical change during a reaction and revert to their original state when the reaction is complete. Alteration of cellular integrity or stimulation of de novo protein synthesis accelerates the release of enzymes into the circulation. The catalytic property is sensitive and specific for each enzyme. The reaction rate catalyzed by each enzyme is proportional to the quantity of the enzyme present. The rate of appearance of a product, disappearance of a substrate, or change in concentration of a coenzyme is usually used as an indirect measure of enzyme quantity. One international unit (IU) is defined as the amount of enzyme that catalyzes the conversion of 1 micromole (microequivalent) of substrate or coenzyme per minute under the defined conditions—specified temperature, optimal pH, defined substrate concentration. The katal (kat) is used to denote catalytic activity; 1 katal represents 1 mole catalyzed per second. One unit equals 16.67 nanokatals.

Temperature and hydrogen ion concentration are optimized and defined for consistency. Change in one or more variables will result in a different value for enzyme activity. Differences in methods among laboratories or in instrumentation may cause different values, both correct, to be determined for an enzyme in aliquots of the same specimen. Within-laboratory measurement drift (variability) is detected by quality control procedures. The appropriate use of quality control specimens that are charted daily, a Levey-Jennings quality control graph, and replicate measurements to evaluate assay precision are essential for detecting inconsistency associated with analytical bias, random analytical variability, and mistakes.

Interpretations associated with the diagnostic use of enzymes are derived from experimental studies, clinical studies, and clinical experience. Certain applications for diagnostic enzymology developed for use in humans were directly incorporated into veterinary medicine without adequate investigation. We now know that some of these diagnostic uses are inappropriate because of species differences. Examples of differences between humans and domestic animals include corticosteroid-associated increases in alkaline phosphatase activity in dogs; bilirubin metabolism in dogs, horses, and cattle; and the paucity of hepatic alanine aminotransferase (ALT) activity in the horses and cattle. Veterinary clinical studies continue to expand and clarify the role of diagnostic enzymology.

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#### 9.1.1 Factors Affecting the Activity of Enzymes (Fig. 8-1; Tables 8-1, 8-2)

Enzymes are located in cells. Some are relatively organ-specific, and others are present in multiple organs. Some that are located in multiple organs have different molecular forms referred to as *isoenzymes* or *isozymes*. They can be separated by electrophoresis on the basis of their differing charges in the pH-adjusted assay media to indicate the specific tissue of origin or subcellular component with which they are associated.

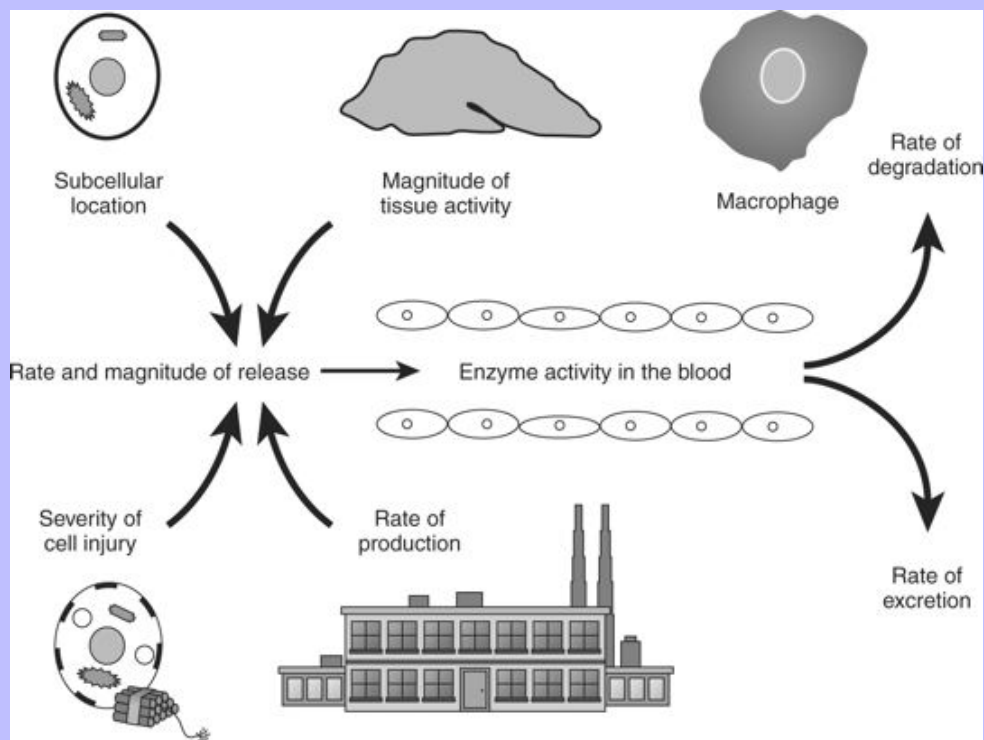
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The location of the enzyme within the cell affects its release. An enzyme may be located in the cytoplasm, attached to organelles, be present in both of these locations, or be attached to an area of the cell membrane. Enzymes present in the cytoplasm are soluble and released with relative ease. They tend to be relatively sensitive markers of altered cellular integrity and can be referred to as *biomarkers* of cell or organ disease. An example is ALT in the cytosol of the hepatocyte. The circulating ALT level increases as a result of altered integrity of the hepatocellular membrane in dogs and cats. An example of the second enzyme location is glutamate dehydrogenase, which is found only in mitochondria of the hepatocyte. Sufficient cell and mitochondrial injury is required for its release. An example of the third situation is aspartate aminotransferase (AST). In the hepatocyte, the majority of AST activity is located in the cytoplasm for most species, and the remainder is associated with mitochondria. Other poorly defined factors may be involved in the release of enzymes from cells. Despite relatively similar relative molecular mass ( $M_r$ ) of approximately 100,000 for both cytosolic ALT and AST, an increase in the circulating ALT level tends to precede the rise in the circulating AST level in dogs after hepatocellular injury. Examples of the fourth cellular enzyme location are alkaline phosphatase and  $\gamma$ -glutamyl transferase, which are expressed by the canalicular membrane of the hepatocyte and biliary system. Impaired bile flow and certain drugs stimulate increased protein synthesis with subsequent release into the circulation.

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Fig. 8-1



The circulating enzyme level is dependent on (1) magnitude of innate tissue activity or ability of an organ to increase its synthesis, (2) cellular location (cytoplasm or attached to organelles), (3) rate and magnitude of release into the circulation as a result of organ disease or enzyme induction, and (4) rate of degradation or excretion.

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The intracellular activity for cytosolic enzymes is usually thousands of times greater than that in the circulation. The circulating enzyme level in health probably reflects a balance between constant leakage through a relatively porous membrane as a result of its dynamic metabolic state and its degradation or excretion from the circulation. It is unlikely that physiologic or “self-programmed” cell death, termed *apoptosis*, has a significant contribution. It appears that most enzymes pass into the interstitial fluid and then into the lymphatic circulation before their appearance in the blood. Their kinetics may be even more complex in disease. For example, studies of pancreatitis have suggested that amylase makes its way from the inflamed organ into the peritoneal fluid, which is followed by the absorption of amylase into the diaphragmatic lymphatic circulation on its way to the peripheral blood.

Table 8-1 Properties and Cellular Location of Diagnostic Enzymes

Enzyme	Cell Location in Liver	Concentration Gradient versus Plasma (Human)
ALT	Cytoplasm	1,025
SDH	Cytoplasm	48,000
GLD	Mitochondria	7,500
AST <sub>1</sub>	Cytoplasm (majority of total tissue enzyme activity)	1,025
AST <sub>2</sub>	Mitochondria	4,375
LD	Cytoplasm	3,000
CK-(total)	Cytoplasm	300
CK-2 (heart)	Cytoplasm	20,000
ALP	Membrane	35
GGT	Membrane and endoplasmic reticulum	35
Cell Location in Pancreas		
IRT	Zymogen granule	
Amylase	Zymogen granule	
Lipase	Zymogen granule	
Modified from Boyd JW: The mechanisms relating to increases in plasma enzymes and isoenzymes in diseases of animals. Vet Clin Pathol 1983;12:9–24, and Pappas Jr NJ: Theoretical aspects of enzymes in diagnosis. Clin Lab Med 1989;9:595–626. ALP, Alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CK-2, isoenzyme 2 (MB); GGT, γ-glutamyl transferase; GLD, glutamate dehydrogenase; LD, lactate dehydrogenase; IRT, immunoreactive trypsin; SDH, sorbitol dehydrogenase.		

Either macrophages or parenchymal cells degrade most enzymes in the circulation. A few are excreted in the urine. Species variation in the elimination of enzymes may affect the diagnostic and investigative uses of enzymes. For example, amylase is excreted in the urine of humans where it can be used as a marker of acute pancreatitis, but amylase appears to be metabolized by the canine kidneys, precluding a similar diagnostic application for dogs. Subtle changes in the physiochemical characteristics of the enzyme can prolong its rate of removal. A macroenzyme represents an enzyme bound to either an immunoglobulin or nonimmunoglobulin component in the circulation. Macroenzymes have been described for most diagnostic enzymes in humans and macroamylasemia in dogs with renal insufficiency. The change in molecular configuration can dramatically prolong a macroenzyme's presence in the circulation, resulting in an increased circulating enzyme level and, consequently, can implicate disease in the organ in which it serves as a biomarker.

The magnitude of increased enzyme level in the circulation is dependent on several factors: the enzyme's innate tissue activity, the severity and type of disease, the rate of enzyme removal from the circulation, and the enzyme's subcellular location.

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Table 8-2 Enzyme Activities in Tissues

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Enzyme	Dog	Cat	Horse	Bovine	Pig	Rat
<b>Alanine aminotransferase</b>						
Liver	32	29.8	0.9	0.3	0.9	2.4
Heart	8.7	1.4	1.7	1.7	3.1	1.7
Muscle	1.8	1.0	4.4	2.0	1.3	2.8
Kidney	2.9	3.7	0.4	0.3	0.5	1.6
Intestine	0.4	0.5	0.03	0.3	—	4.5
Pancreas	1.5	0.9	0.7	—	—	—
<b>Sorbitol dehydrogenase</b>						
Liver	11.7	0.60	5.2	9.7	9.5	31
Heart	1.0	0.20	0.2	0.1	0.00	0
Muscle	0.4	0.05	0.0	0.1	0.03	0
Kidney	6.5	0.10	1.0	3.0	3.70	11
Intestine	0.7	0.04	0.1	0.4	0.20	0
Pancreas	0.4	0.06	0.2	—	0.00	—
<b>Glutamate dehydrogenase</b>						
Liver	6.8	125	10.4	19.0	1.3	36
Heart	2.3	10.0	0.1	2.1	0.1	3
Muscle	0.6	2.0	0.1	0.7	0.01	2.4
Kidney	5.1	40	0.7	8.5	0.3	7.9
Intestine	2.0	3.0	—	1.2	—	3.3
Pancreas	0.5	—	0.3	—	—	—
<b>Aspartate aminotransferase</b>						
Liver	53	59	33	70	57	88
Heart	67	69	32	48	115	269
Muscle	46	22	54	70	44	69
Kidney	27	17	7	30	47	57
Intestine	12	7	6	6	—	25
Pancreas	22	10	—	—	—	—
<b>Lactate dehydrogenase</b>						
Liver	130	127	82	57	25	212
Heart	320	89	354	97	60	360
Muscle	169	259	155	336	591	453
Kidney	256	40	122	69	30	167
Intestine	58	47	18	20	29	185
Pancreas	52	16	52	—	12	—
<b>Creatine kinase</b>						
Liver	50	1	7	—	—	10
Heart	1150	518	1710	—	—	644
Muscle	2500	692	4300	—	—	3516
Kidney	50	1	97	—	—	31
Intestine	200	20	257	—	—	223
Pancreas	—	15	—	—	—	—
<b>Alkaline phosphatase</b>						
Liver	0.3	0.30	0.74	0.09	0.3	1.17
Heart	0.8	0.05	0.07	0.01	0.1	—
Muscle	0.1	0.05	0.10	0.02	0.1	0.5
Kidney	6.4	3.79	10.20	0.80	13.0	300
Intestine	220	7.98	2.24	—	10.1	—
Pancreas	1.3	0.19	2.15	0.07	—	0.6
<b>γ-Glutamyl transferase</b>						

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Liver	0.90	—	2.40	4.97	2.69	0.13
Heart	—	—	0.12	0.31	0.02	0
Muscle	0.00	—	0.28	0.01	0.02	0
Kidney	86.10	—	38.66	60.50	16.45	189
Intestine	1.50	—	0.59	0.59	1.47	—
Pancreas	41.70	—	6.80	22.18	8.58	—

Modified from Boyd JW: The mechanisms relating to increases in plasma enzymes and isoenzymes in diseases of animals. Vet Clin Pathol 1983;12:9–24.

Measurements listed in U/g throughout.

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## 9.2 PHYSIOLOGIC CONSIDERATIONS (Table 8-3)

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Selected laboratory test results that are normal for growing animals are outside the reference range for adults. The increased concentration of growth hormone is, at least in part, responsible for the increased circulating level of phosphate. Skeletal growth causes an increased circulating level of alkaline phosphatase as a result of increased activity of the bone isoenzyme. Excitement or exercise-induced epinephrine release and stress-induced accelerated release of corticosteroids can cause changes in biochemical analyte values. A transient hyperglycemia with or without a transient glucosuria can occur, especially in cats. Chronic stress in the dog may cause an increase in the circulating level of alkaline phosphatase. The hydration state will affect expressed concentration or activity of an analyte because of the reduced water content of the plasma. Dilutional changes are expected for serially monitored measurements during rehydration, notably total protein. Hemoconcentration caused by dehydration results in an improper citrate-to-blood ratio, which will prolong the activated partial thromboplastin time. Diet can alter several biochemical measurements. A high-protein diet can increase the serum urea nitrogen concentration. The prolonged use of a low-protein diet can cause hypoalbuminemia or a reduction in the circulating blood urea nitrogen (BUN) level.

## 9.3 SPECIMEN INTERFERENCES (See Table 8-3)

Lipemia causes a number of changes in hematology and chemistry measurements by light scattering in spectrophotometric methods. Refractometer readings of the plasma protein concentration will be increased and cause a discrepancy between it and the total serum protein value measured biochemically. The serum electrolyte values measured by flame photometry are decreased, whereas those measured by ion-specific electrodes are not affected. The lipid can be partially cleared by ultracentrifugation techniques or precipitating agents, although clearing agents may themselves induce artifacts. The removal of the lipid may in itself adversely affect the validity of the test value. Lipoproteins bind bile acids, which would be discarded along with the lipid after ultracentrifugation. Lipemia can cause an increased bilirubin value to be measured by commonly used methods. If the bilirubin value is greater than 1.0 mg/dL, it is often associated with concomitant bilirubinuria in dogs. If the value is greater than 2.5 to 3.0 mg/dL, it is expected to be associated with icteric tissues in most species. If these relationships are not clinically present, the validity of the bilirubin value is questionable. Lipemia predisposes to hemolysis in vitro, which may further confound the effect on the test value. The best approach is to visually examine the sample for lipemia and hemolysis before its submission. If lipid is present on visual inspection, discarding the sample and obtaining a second one will preclude any chance of misinformation. An overnight fast will usually be adequate preparation for obtaining the second sample. The occurrence of lipemia after a fast may be in itself indicative of disease.

Hemolysis can directly interfere with the spectrophotometric absorbance reading and alter the pH of enzymatic reactions. AST and lactate dehydrogenase have higher activity within erythrocytes compared with the circulating level, and their levels will be increased after in vivo or in vitro hemolysis. A mean cell hemoglobin concentration that is greater than the reference range is a numerical indication of hemolysis. Moderate to marked hemolysis is

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accompanied by hemoglobinuria if it is an in vivo phenomenon. The absence of hemoglobinuria suggests in vitro lysis of erythrocytes as a result of sample collection.

Bilirubin increases the concentration of albumin determined by the 2-*p*-hydroxyphenylazobenzoic acid (HABA) procedure, the cholesterol concentration when ferric chloride reagents are used, the glucose concentration when the *o*-toluidine method is used, and the total protein concentration as measured by the biuret method. Severe hyperbilirubinemia can decrease the serum creatinine level (Jaffe reaction). Bile acids can adversely affect the measurements for serum secretin, glucagon, insulin, and gastrin determined by radioimmunoassays. Marked hyperglobulinemia can increase the phosphate value and decrease the serum electrolyte measurements obtained by flame photometry. Hyperglobulinemia does not affect the ion-specific electrode measurement of these values.

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Heparin is an acceptable anticoagulant for most chemistry, blood gas, and ammonia determinations. Notable exceptions include ammonium heparin for the measurement of ammonia and sodium and potassium heparin for the measurement of those respective electrolytes.

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**Table 8-3 Effect of Various Factors on the Measurement of Constituents in Serum or Plasma**

Variables	Analyte																			
	BUN	Creatinine	ALT	AST	ALP	GGT	Bilirubin	Acids	Glucose	Cholesterol	Triglycerides	Calcium	Phosphorus	Protein	Globulins	Albumin	Sodium	Chloride	Potassium	Magnesium
Lipemia	V	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Hemolysis	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Icterus	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Hyperglycemia	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Hyperproteinemia	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Ketoneuria	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Severe azotemia	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Immature animals	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Aging changes	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
(3-12 yr)	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Anticoagulants	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
EDTA	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Oxalate	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Citrate	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Fluoride	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Heparin	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
Sample dehydration	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u
UV light	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u

†, Value increased because of physiologic change; ↓, value decreased because of physiologic change; u, value increased because of interference with method or collection changes; u, value decreased because of interference with method or collection changes; V, variable changes depending on method; I, flame photometric methods only; ion-specific electrodes not affected; 2, radio-immunoassay not affected; 3, dry reagent methods; 4, Akita dogs; 5, potassium salt; 6, sodium; 7, refractometer; 8, ammonium; 9, if nursing. ALP, alkaline phosphatase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; EDTA, ethylenediaminetetraacetic acid; GGT, γ-glutamyl transferase; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; PCV, packed cell volume.

## 9.4 PHARMACOLOGIC AND THERAPEUTIC AGENTS (Tables 8-4, 8-5)

Topical, oral, or parenteral corticosteroids can cause changes in the hemogram, biochemistry profile, urinalysis, and endocrine test results. Corticosteroids can cause abnormal liver test results in dogs and may impair the renal concentrating mechanism, resulting in dilute urine. Corticosteroids such as prednisone are measured in cortisol assays, resulting in an erroneously increased value. Corticosteroids may suppress the hypothalamic-pituitary-adrenal axis, which causes a reduction in the circulating cortisol level. Aspirin and acetaminophen artificially decrease serum glucose values measured by the oxidase system. Intravenous dipyrone can negatively interfere with measurement of creatine kinase, lactate dehydrogenase, triglyceride, cholesterol, and creatinine concentrations. Anticonvulsant medications can cause increased liver enzyme levels, especially in dogs. The bromide in potassium bromide is measured as chloride by flame photometry and ion-specific electrode methods. The administration of ascorbic acid may cause a reduced glucose value to be measured (oxidase method) or may cause false-negative glucose and nitrate reactions on the urine reagent strip. Supplements containing cobalamin (vitamin B<sub>12</sub>) and folate (folic acid) will increase the measured circulating levels of vitamin B<sub>12</sub> and folic acid, which precludes their diagnostic use for assessment of intestinal disease.

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Table 8-4 Effect of Drugs or Other Factors on the Measurement of Constituents in Urine

Drug or Variable	Urinalysis									
	Specific gravity	Urine pH	Proteinuria (Dipstick)	Glucosuria (Dipstick)	Ketoneuria	Bilirubinuria	Urobilinogen	Hemo/Myoglobinuria	Nitrituria	Pyuria
Acetazolamide	↑		↑1				↑			
Aminoglycosides			↑2	↑						↑1
Ascorbic acid	↓			↓		↓	↓		↓	
Cephalosporins			↑2							↑1
Chlorpromazine			↑2			↓				
Colchicine	↓									
Corticosteroids	↓									
Dipyrene				↓						
Diuretics	↓									
Methionine	↓			↑						
Penicillins			↑2							
Phenazopyridine			↑1		↑	↑	↑		↑	
Phenolphthalein				↑						
Phenothiazines					↑	↑	↑			
Procaine							↑			
Radiographic contrast media	↑		↑2							
Salicylates			↑2	↓		↓				
Sodium bicarbonate	↑		↑1				↑			
Sulfobromophthalein (BSP)					↑		↑			
Sulfonamides			↑2				↑			
Urinary acidifiers	↓						↓			
Acetoacetate (ketonuria)				↓						
Alkaline urine	↓1		↑1 ↓2						↓3	
Bilirubinuria							↑			
Highly concentrated urine								↓		
Nitrituria						↓	↓	↓		
Proteinuria	↑1									
Refrigerated urine				↓						
Time		↑								
UV light						↓	↓			

↑, Value increased because of physiologic change; ↓, value decreased because of physiologic change; ↑, value increased because of interference with method; ↓, value decreased because of interference with method or collection changes; 1, dipstick; 2, sulfosalicylic acid method; 3, sediment.

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Table 8-5 Effect of Drugs or Hormones on the Measurement of Constituents in Serum and Plasma

Drug or Hormone	Analyte																									
	BUN	Creatinine	ALT	AST	ALP	GGT	Bilirubin	Acids	Glucose	Cholesterol	Triglycerides	Calcium	Phosphorus	Total Protein	Albumin	Sodium	Chloride	Potassium	Magnesium	Amylase	Lipase	CK	Thyroxine	ACTH	Cortisol	Gastrin
Corticosteroids	↑		↑	↑	↑	↑		↑						↑												
Mineralocorticoids						↑												↓								
Anabolic steroids														↑							↑	↑	↑		↓	
NSAIDs	↑	↑									↓							↑								
Acetaminophen			↑				↑		↓									↓								
Aspirin	↑	↑	↑					↓											↓							
Dipyron		↓							↓		↓												↓			
Ibuprofen	↑	↓		↑																						
Phenylbutazone	↑						↑																			
Antibiotics																		↑								
Aminoglycosides	↑																									
Cephalosporins	↓						↑																			
Chloramphenicol																										
Penicillins	↓																									
Sulfonamides			↑	↑	↑	↑																	↑	↑		
Tetracycline													↓									↑	↑			
Anticonvulsants												↓	↓													
Phenobarbital			↑	↑	↑	↓																			↑	
Phenytoin			↑	↑				↑		↑																
Potassium bromide									↓									↓								
Primidone																										
Hormones			↑	↑																						
Androgens																										
Estrogen															↓			↑								
Insulin											↑		↓											↑	↑	
Progesterone																										
Thyroxine								↑		↓													↑			
Amphotericin B	↑																	↓		↓						
Ascorbic acid		↓				↓		↓																		
Asparaginase									↑	↓																
Azathioprine																							↑			
Barbiturates																						↑	↑			
β-Adrenergics		↓		↑																						
Captopril																										
Cholestyramine	↑	↑						↓		↓								↑								
Cimetidine																										
Cisplatin	↑	↑																								
Colchicine										↓																
Flucytosine		↓																								
Furosemide	↑																									
Glucose		↓																								
Heparin											↓															
Methimazole																										
Metronidazole																										
Phenothiazines																										
Propranolol																										
Radiographic contrast media	↑	↑																								
Salicylates	↑	↑		↑	↑				↓																	
Sodium bicarbonate																										
Sulfobromophthalein (BSP)	↓																	↑		↓	↓					
Theophylline																										
Thiacetarsamide			↑						↑																	

↑, Value increased because of physiologic change; ↓, value decreased because of physiologic change; ↓, value decreased because of interference with method or collection changes; ↓, variable changes depending on methodology; ACTH, adrenocorticotrophic hormone; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; GGT, γ-glutamyl transferase; NSAIDs, nonsteroidal antiinflammatory drugs

## 9.5 COLLECTION AND STORAGE (See Table 8-3)

Prolonged contact of erythrocytes with serum will decrease the serum glucose concentration at a rate of about 10% per hour. Most biochemical values are stable in serum stored at 4°C for at least 24 hours. Bilirubin in serum or urine is oxidized to biliverdin with prolonged exposure to fluorescent lightening. Some hormones are adversely affected by prolonged or inappropriate storage or by the type of collection tube. The laboratory should be contacted when hormones such as adrenocorticotrophic hormone, parathyroid hormone, insulin, and gastrin are measured. Sample evaporation during transportation can cause an increase in biochemical values; noteworthy are total protein, sodium, and potassium concentrations. In an arid environment, dehydrated sample-related increases in these values could be interpreted as clinically important after only 2 hours of exposure to the dry air. Bacterial growth in a serum collection tube can cause a decreased glucose value if processing is delayed without refrigeration.

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## 9.5.1 Vitreous and Aqueous Humor Specimens

Postmortem blood undergoes rapid chemical change and is easily contaminated, precluding its diagnostic use. Selected biochemical constituents can be measured in the vitreous or aqueous humor after death for diagnostic purposes. In canine vitreous humor, the increase of the potassium concentration is dependent on time and temperature and can be used to assess the time of death. The chloride concentration is stable for up to 24 hours at both 4°C and 20°C. The sodium concentration fluctuates within this time and temperature range, but increased values suggest antemortem hypernatremia. Creatinine and BUN levels are stable for up to 24 hours at both 4°C and 20°C. The glucose concentration decreases by approximately 50% within 3 hours at both temperatures and by approximately 75% of the antemortem concentration by 24 hours. A normal or increased value in the vitreous humor would suggest antemortem hyperglycemia. In bovine vitreous humor, the magnesium, sodium, and chloride concentrations are stable at ambient temperature for up to 24 hours after death. The BUN and creatinine levels in bovine or equine vitreous humor are reliable indicators of azotemia for up to 24 hours after death at ambient temperature.  $\gamma$ -Glutamyl transferase is stable for up to 4 hours at ambient temperature in rabbit aqueous humor.

## 9.6 ADDITIONAL READING

- M Burkhard, D Meyer: Causes and effects of interference with clinical laboratory measurements and examinations. In Kirk, R, Bonagura, J (Eds.): *Current veterinary therapy XII*. 1995, WB Saunders Co, Philadelphia, 14–20.
- GH Cantor, GH Palmer, BW Fenwick: Analysis of post mortem aqueous humor chemistry in the horse, with particular reference to urea nitrogen and creatinine. *Equine Vet J*. **21**, 1989, 288–291.
- S Ceryak, B Bouscarel, H Fromm: Comparative binding of bile acids to serum lipoproteins and albumin. *J Lipid Res*. **34**, 1993, 1661–1674.
- A Chauvet, E Feldman, P Kass: Effects of phenobarbital administration on results of serum biochemical analyses and adrenocortical function tests in epileptic dogs. *J Am Vet Med Assoc*. **207**, 1995, 1305–1307.
- M Corazza, R Tognetti, G Guidi, et al.: Urinary  $\alpha$ -amylase and serum macroamylase activities in dogs with proteinuria. *J Am Vet Med Assoc*. **205**, 1994, 438–440.
- S Dial: Hematology, chemistry profile, and urinalysis for pediatric patients. *Comp Contin Educ Small Anim Pract*. **14**, 1992, 305–308.
- M Fettman, T Allen: Developmental aspects of fluid and electrolyte metabolism and renal function in neonates. *Comp Cont Educ Small Anim Pract*. **14**, 1991, 392–403.
- B Gerstman, D Cappucci: Evaluating the reliability of diagnostic test results. *J Am Vet Med Assoc*. **188**, 1986, 248–251.
- R Grasbeck: Reference values, why and how. *Scand J Clin Lab Invest*. **201**, 1990, 45–53.
- I Hall, K Campbell, M Chambers, et al.: Effect of trimethoprim/sulfamethoxazole on thyroid function in dogs with pyoderma. *J Am Vet Med Assoc*. **202**, 1993, 1959–1962.
- JA Halsted, CH Halsted: In *The laboratory in clinical medicine, interpretation and application*. 1981, WB Saunders Co, Philadelphia.
- W Hoffmann, J Dorner: Disappearance rates of intravenously injected canine alkaline phosphatase isoenzymes. *Am J Vet Res*. **38**, 1977, 1553–1556.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

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- Y Kamimoto, S Horiuchi, S Tanase, et al.: Plasma clearance of intravenously injected AST isozymes: Evidence for preferential uptake by sinusoidal liver cells. *Hepatology*. **5**, 1985, 367–375.
- M Kuhlenschmidt, W Hoffmann, M Rippey: Glucocorticoid hepatopathy: Effect on receptor-mediated endocytosis of asialoglycoproteins. *Biochem Med Metab Biol*. **46**, 1991, 152–168.
- G Lake-Bakaar, S Tovoli, E Straus, et al.: The effects of bile salts on the radioimmunoassay of hormonal peptides. *J Lab Clin Med*. **99**, 1982, 740–745.
- D Lee, S Lamb, T Reimers: Effects of hyperlipemia on radioimmunoassays for progesterone, testosterone, thyroxine, and cortisol in serum and plasma samples from dogs. *Am J Vet Res*. **52**, 1991, 1489–1491.
- S Lekhakula, S Boonpisit, B Amornkitticharoen: Total bile acids in hyperlipidaemic serum determined by bioluminescent and spectrophotometric methods. *J Biolumin Chemilumin*. **6**, 1991, 259–262.
- SD Lincoln, VM Lane: Postmortem chemical analysis of vitreous humor as a diagnostic aid in cattle. *Mod Vet Pract*. **9**, 1985, 883–886.
- J Lott, L Mitchell, M Moeschberger, et al.: Estimation of reference ranges: How many subjects are needed?. *Clin Chem*. **38**, 1992, 648–650.
- L Lowseth, N Gillett, R Gerlach, et al.: The effects of aging on hematology and serum chemistry values in the beagle dog. *Vet Clin Pathol*. **19**, 1990, 13–19.
- JT Lumeij: Fasting and postprandial plasma bile acids concentrations in racing pigeons and Mallards. *J Assoc Avian Vet*. **5**, 1991, 197–200.
- Pappas, N Jr.: Theoretical aspects of enzymes in diagnosis. *Why do serum enzymes change in hepatic, myocardial, and other diseases?* *Clin Lab Med*. **9**, 1989, 595–626.
- Pappas, N Jr., A Qureshi: Liver AST activity as a power function of body weight. *Biochem Med Metab Biol*. **39**, 1988, 121–125.
- T Reimers, S Lamb, S Bartlett, et al.: Effects of hemolysis and storage on quantification of hormones in blood samples from dogs, cattle, and horses. *Am J Vet Res*. **52**, 1991, 1075–1080.
- A Remaley, P Wilding: Macroenzymes: Biochemical characterization, clinical significance, and laboratory detection. *Clin Chem*. **35**, 1989, 2261–2270.
- N Saris: Approved recommendation (1978): Quantities and units in clinical chemistry. *J Clin Chem Clin Biochem*. **17**, 1979, 807–821.
- P Schoning, AC Straffuss: Postmortem biochemical changes in canine vitreous humor. *J Forensic Sci*. **25**, 1980, 53–59.
- P Solter, W Hoffmann, J Hoffman: Evaluation of an automated serum bile acids assay and the effect of bilirubin, hemoglobin, and lipid on the apparent bile acid yield. *Vet Clin Pathol*. **21**, 1992, 114–118.
- B Statland, P Winkel: Preparing patients and specimens for laboratory testing. In Henry, J (Ed.): *Clinical diagnosis and management by laboratory methods*. 18th ed., 1991, WB Saunders Co, Philadelphia, 68–71.
- Y Sugimoto, T Hayakawa, T Kondo, et al.: Peritoneal absorption of pancreatic enzymes in bile-induced acute pancreatitis in dogs. *J Gastroenterol Hepatol*. **5**, 1990, 493–498.
- S Thoresen, G Havre, H Morberg, et al.: Effects of storage time on chemistry results from canine whole blood, heparinized whole blood, serum and heparinized plasma. *Vet Clin Pathol*. **21**, 1992, 88–94.
- N Tietz: In *Clinical guide to laboratory tests*. 2nd ed., 1990, WB Saunders Co, Philadelphia.
- P Wolf: The significance of transient hyperphosphatasemia of infancy and childhood to the clinician and clinical pathologist. *Arch Pathol Lab Med*. **119**, 1995, 774–775.

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## 10 Chapter 9 Evaluation of Plasma Proteins

The fluid in which blood cells circulate is called *plasma*. It is prepared in the laboratory by collecting blood with an anticoagulant, followed by centrifugation to remove the blood cells. If blood is collected without anticoagulant and allowed to clot, the fluid that is obtained after centrifugation is called *serum*. The protein concentration in plasma is usually about 0.2 to 0.5 g/dL higher than that in serum, primarily because of the presence of fibrinogen in plasma, which is consumed during coagulation.

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Hundreds of proteins with a wide variety of functions circulate in plasma. With the exception of immunoglobulins, most proteins are synthesized by the liver. Some are transport proteins that are vital to the movement of nutrients, hormones, and metabolic waste products through the blood. Plasma proteins exhibit important colloid osmotic effects required for maintaining normal blood volume, and they account for about 15% of the acid-buffering capacity of blood. Immunoglobulins and acute-phase proteins (APPs) are important in immunity. Certain plasma proteins are essential for hemostasis; they have important functions in platelet adhesion and aggregation, as well as in coagulation.

The total plasma protein concentration is low at birth in domestic mammals (4 to 6 g/dL) but increases after the absorption of immunoglobulins from colostrum. The total plasma protein concentration continues to increase gradually with age as a result of immunoglobulin production in response to foreign antigens. Normal adult mammal plasma protein concentrations vary by species but are generally between 6 and 8 g/dL.

Total proteins are easily measured in plasma, serum, and body cavity fluids by using hand-held refractometers. The bending (refraction) of light as it passes through the sample is proportional to the total amount of dissolved solids present in solution. Proteins cause most of the refraction of light in plasma. The amount of nonprotein solids (e.g., electrolytes, glucose, lipids, and urea) present in plasma and other body fluids is relatively constant; consequently, after subtraction of nonprotein solids (about 1.5 g/dL), total protein scales are displayed in the refractometer.

Different brands of refractometers provide somewhat different results, with American-made refractometers generally producing values 0.3 g/dL to 0.5 g/dL higher than Japanese-made refractometers. Increased concentrations of nonprotein solids such as lipids, urea, glucose, and exogenous substances can result in erroneously increased total plasma and total serum protein concentrations. Mild hemoglobinemia and hyperbilirubinemia are not major causes of error.

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Total proteins in plasma and serum are also measured biochemically as part of clinical chemistry panels with the biuret method. However, this method is not sensitive enough for the measurement of cerebrospinal fluid protein concentrations. Lipemia, hyperbilirubinemia, and marked hemoglobinemia can result in erroneously increased total plasma protein concentrations measured when the biuret method is used.

In addition to spurious causes cited previously, total plasma (or serum) protein concentrations can be increased (hyperproteinemia) by dehydration or by increased synthesis of globulins. Low total plasma (or serum) protein concentrations (hypoproteinemia) may result from overhydration; decreased production of albumin or immunoglobulins; or protein loss associated with hemorrhage, vasculitis or vessel injury, protein-losing nephropathies, or protein-losing enteropathies. The hematocrit should also be low if the hypoproteinemia results from overhydration or hemorrhage (see [Chapter 2](#)). Except for hemorrhage in which albumin and globulins are lost together, albumin may be preferentially lost relative to globulins in some protein-losing disorders because albumin molecules are smaller than most other plasma proteins.

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## 10.1 CLASSIFICATION OF PLASMA PROTEINS

Plasma proteins are classified in various ways. They are divided into albumin and globulins (all other plasma proteins) by using clinical chemistry analyzers. Total protein and albumin concentrations are measured directly in biochemical assays, and the total globulin concentration is determined by subtracting the albumin concentration from the total protein concentration. The total globulin concentration in plasma is slightly higher than that in serum because of the presence of fibrinogen in plasma.

Most plasma proteins carry a negative charge when dissolved in alkaline buffers for electrophoresis. When applied to cellulose acetate strips or agarose gels, plasma proteins can be separated on the basis of their charge densities and resultant mobilities in an electric field. Albumin, a protein of low molecular weight and high negative charge, migrates with the greatest velocity toward the anode (positive pole). The negative charge density of the various globulins decreases from  $\alpha$ -globulins to  $\beta$ -globulins. Depending on the pH of the buffer, the  $\gamma$ -globulins may have minimal negative charge, moving little from the point of application, or some may be positively charged and moving toward the cathode (negative pole). Serum is generally used for electrophoresis rather than plasma, because the fibrinogen present in plasma migrates between the  $\beta$ -globulins and  $\gamma$ -globulins, complicating their differentiation and interpretation. After electrophoresis, the membrane or gel is stained with a protein stain such as Coomassie blue or Ponceau S and scanned by using a densitometer. The width and density of each protein band are represented by the width and height of a peak on the densitometer tracing (Fig. 9-1). This tracing or graph is sometimes called an *electrophoretogram*. As an aid to identifying protein peaks on electrophoretograms, the halfway point between the beginning and end of the electrophoretogram generally lies between the  $\alpha_2$ -globulins and  $\beta$ -globulins, and the midpoint between the beginning of the  $\beta$ -globulins and the end of the  $\gamma$ -globulins generally separates the  $\beta$ -globulins and  $\gamma$ -globulins. Two or three protein peaks may be identified in the  $\alpha$  and  $\beta$  regions, but usually only one broad peak is present in the  $\gamma$  region (Fig. 9-2). The area under each peak of the densitometer tracing is measured, the percentage of each protein band is determined, and a concentration for each protein band is calculated by multiplying the percentage of each band (expressed as a fraction) times the total serum protein concentration determined by chemical assay. With the exception of the albumin peak, each peak is composed of a variety of different proteins, which may be separated by more complicated immunoelectrophoretic techniques or specific biochemical assays.

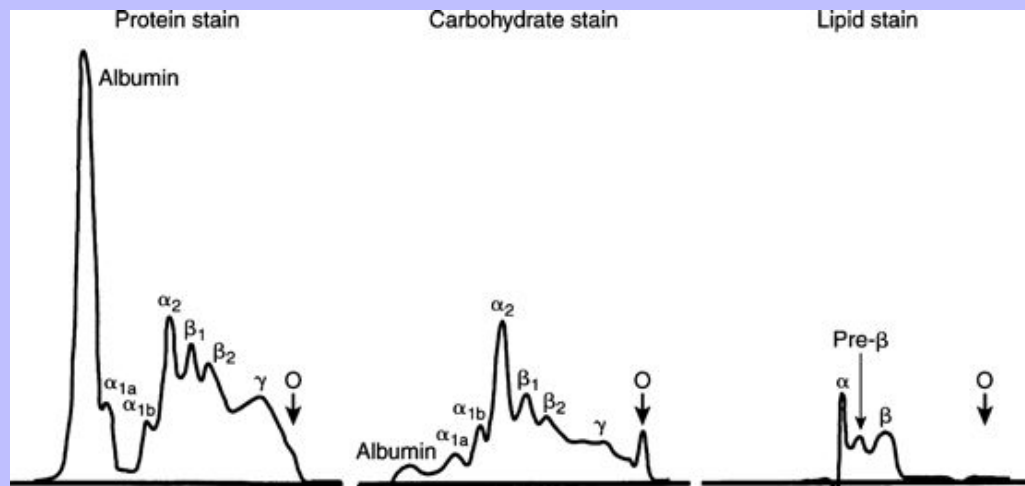
Proteins are sometimes classified by the amount of carbohydrate (glycoprotein) or lipid (lipoprotein) that they contain. Nearly all plasma proteins have some oligosaccharide groups attached to a protein core. However, proteins containing significant amounts of carbohydrates have been classified as glycoproteins. When serum proteins are stained for carbohydrate after electrophoresis (see Fig. 9-1), the pattern seen is considerably different from that observed when a protein stain is used. When serum proteins are stained for lipids after electrophoresis (see Fig. 9-1), the pattern is different from that observed when a protein or carbohydrate stain is used. These lipoproteins are complexes of lipids and proteins called *apolipoproteins*.

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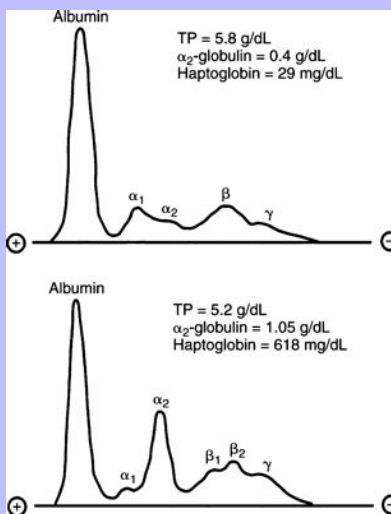
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Fig. 9-1



Densitometer tracings of electrophoresis performed on a serum sample from a healthy pony stained for protein (left tracing), carbohydrate (middle tracing), and lipids (right tracing). O represents origin where proteins were placed before electrophoresis. (Redrawn from Rollins JB, Shultz TD, Fiser RH: Serial measurements of serum protein, glycoprotein, and lipoprotein fractions in normal and Venezuelan equine encephalomyelitis-vaccinated ponies and burros. *Am J Vet Res* 1972;33:323-327.)

Fig. 9-2



Serum protein electrophoretograms from two dogs. The one at the top is from a dog with a normal serum protein electrophoretogram and low-normal haptoglobin concentration. The one at the bottom is from a dog with hepatic necrosis. The  $\alpha_2$ -globulin and haptoglobin concentrations are increased. TP, Total protein.

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## 10.2 ALBUMIN

Albumin is a single, homogeneous plasma protein that contains small amounts of carbohydrate. Albumin and other proteins can become glycosylated by nonenzymatic interactions with glucose. Glycosylated albumin and total glycosylated proteins (which can be measured as fructosamine) are increased in humans and animals with untreated or poorly controlled diabetes.

Albumin concentration varies by species but is generally between 2.5 g/dL and 4.5 g/dL in plasma or serum. Albumin has a smaller molecular weight and moves further on electrophoresis gels than do the globulins, because albumin has a high negative charge density. Albumin is the most important plasma protein in the maintenance of osmotic pressure of blood because osmotic pressure depends on the number of molecules present. Although the albumin concentration in plasma is similar to the total globulin concentration, there are many more albumin molecules present in plasma than there are globulin molecules, because albumin molecules are smaller (molecular weight of approximately 68 kd) than most globulin molecules. Albumin is also an important transport protein in blood, binding many organic and inorganic substances not transported by specific proteins. Examples of substances bound by albumin include free cations such as calcium, copper, and zinc; metabolites such as bilirubin and free fatty acids; certain hormones; poorly soluble drugs; and various toxic substances. In contrast to primates, in domestic animals, plasma albumin does not bind free heme with an affinity high enough to form methemalbumin.

Plasma (or serum) albumin concentrations can be directly measured biochemically by using dye-binding assays (generally bromocresol green in animals) or by calculation after protein electrophoresis. Unfortunately, albumins do not bind dyes equally by weight in all species; consequently, erroneously low or high albumin concentrations may be measured in a species for which the albumin assay has not been calibrated. Albumin concentration may be overestimated in heparinized plasma samples, depending on species and assay conditions used.

In addition to assay errors, low plasma albumin concentrations (hypoalbuminemia) may occur as a result of overhydration, loss of albumin from the body (hemorrhage, protein-losing nephropathies, protein-losing enteropathies, profound exudation), sequestration within peritoneal and/or pleural cavities or subcutaneous tissues (increased intravascular hydrostatic pressure or vasculopathies), decreased albumin synthesis associated with chronic hepatic insufficiency (synthetic liver failure), and severe malnutrition (inadequate protein intake, digestion, or absorption). Albumin is a negative APP; consequently, a mild hypoproteinemia is often found in inflammatory conditions. Albumin synthesis is also decreased in response to hyperglobulinemia. Finally, hypoalbuminemia has been reported in about one third of dogs with hypoadrenocorticism, but the pathogenesis of the hypoalbuminemia is unknown. There are no disorders in which albumin synthesis is increased; consequently, increased albumin concentration suggests that dehydration or an assay error is present. Alterations in albumin concentration are most accurately interpreted when they are examined in light of the total globulin concentration (Box 9-1).

## 10.3 GLOBULINS

The total globulin concentration is calculated in plasma or serum by subtracting the measured albumin concentration from the total protein concentration measured with the biuret method. Consequently, an error in either the total protein or albumin measurements can result in an erroneous total globulin concentration. The globulins are a very heterogeneous group of proteins that can be classified as  $\alpha$ ,  $\beta$ , or  $\gamma$  globulins by electrophoresis. Their functions vary greatly, and some are discussed in the following sections.

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## 10.3.1 BOX 9-1 Concomitant Interpretation of Albumin and Total Globulin Concentrations

### 10.3.1.1 Normal albumin with:

*Low globulins*—Failure of passive transfer in neonates, acquired or inherited defects of immunoglobulin synthesis

*Normal globulins*—Normal

*High globulins*—Increased globulin synthesis, dehydration-masked hypoalbuminemia

### 10.3.1.2 High albumin with:

*Low globulins*—Assay error resulting in spuriously high albumin concentration

*Normal globulins*—Dehydration-masked hypoglobulinemia

*High globulins*—Dehydration

### 10.3.1.3 Low albumin with:

*Low globulins*—Substantial ongoing or recent blood loss, overhydration, massive exudation, protein-losing enteropathy

*Normal globulins*—Protein-losing nephropathy, terminal liver disease, malnutrition, hypoadrenocorticism in dogs, vasculopathy (e.g., endotoxemia, septicemia, Rocky Mountain spotted fever, immune-mediated vasculitis), increased hydrostatic pressure (e.g., portal hypertension, right-sided congestive heart failure), peritoneal dialysis

*High globulins*—Inflammation, multiple myeloma, lymphoproliferative diseases

Patterns shown are typical, but overlaps can occur. For example, some inflammatory vasculopathies (vasculitis) may have increased globulins, and some protein-losing enteropathies may have hypoalbuminemia without hypoglobulinemia.

The total plasma globulin concentration may be low (hypoglobulinemia) because of overhydration, loss of globulins from the body (hemorrhage, massive exudation, protein-losing enteropathy), a failure of passive transfer of immunoglobulins in neonatal animals, or a defect in immunoglobulin synthesis. Total globulin values may be artifactually low if a technical error results in a spuriously high albumin concentration, because total globulin concentration is determined by subtracting the albumin concentration from the total protein concentration in the sample (see [Box 9-1](#)).

Hyperglobulinemia may occur because of dehydration or increased globulin synthesis. Increased synthesis of APPs may contribute to hyperglobulinemia that occurs in association with inflammatory responses to tissue injury and/or foreign antigens, but increased immunoglobulin synthesis is generally required for the total globulin concentration to exceed the reference interval. Neoplastic B lymphocytes and plasma cells may also exhibit increased immunoglobulin synthesis. Alterations in globulin concentration are most accurately interpreted when

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they are examined with total albumin concentration (see [Box 9-1](#)) and with results from serum protein electrophoresis (discussed later in this chapter).

Table 9-1 Species Differences in Acute-Phase Protein Responses

Protein	Major	Moderate	Minor	Negative
Fibrinogen		All species		
Haptoglobin	Cattle, sheep	Dogs, cats, pigs, horses		
Serum amyloid A	Dogs, cats, horses, cattle, pigs			
C-reactive protein	Dogs	Horses, pigs		
$\alpha_1$ -Acid glycoprotein		Dogs, cats, horses, cattle, sheep		
Ceruloplasmin			Dogs, horses, pigs, cattle	
Albumin				All species
The acute-phase proteins (APPs) that increase only two to four times normal concentrations are considered <i>moderate APPs</i> and those that exhibit many-fold higher increases are termed <i>major APPs</i> .				

### 10.3.2 Acute-Phase Proteins

Some globulins are termed *acute-phase proteins* because their concentrations rapidly increase as part of an acute-phase response to infection, inflammation, and tissue injury. Inflammatory cytokines including interleukin-1, interleukin-6, and tumor necrosis factor- $\alpha$  initiate the acute-phase response, which includes increased synthesis of positive APPs by the liver. The acute-phase response, a part of the innate immune system, also includes fever, neutrophilia, decreased serum iron concentration, muscle protein loss, and hormone alterations that combine to protect the body and enhance the repair process after injury. The magnitude of increase in APP concentrations varies by protein and species ([Table 9-1](#)). The APPs that increase only two to four times normal concentrations are considered moderate APPs, and ones that exhibit many-fold increases are considered major APPs.

### 10.3.3 $\alpha_1$ -Acid Glycoprotein

$\alpha_1$ -Acid glycoprotein (orosomucoid, seromucoid) is an important drug-binding protein that binds a variety of drugs with different affinities than those bound by albumin. Its molecular weight is about 40 to 50 kd, with carbohydrate making up 30% or more of the molecule in some species.  $\alpha_1$ -Acid glycoprotein is an APP that increases in response to inflammation and neoplasia. It is also reported to increase about fourfold in plasma of dogs treated with phenobarbital.

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### 10.3.4 $\alpha_1$ -Protease Inhibitor

$\alpha_1$ -Protease inhibitor ( $\alpha_1$ -antitrypsin) is the main plasma protein in the  $\alpha_1$  region on serum protein electrophoresis. This approximately 60-kd protein is an important inhibitor that protects against proteases released from phagocytes during inflammatory processes.  $\alpha_1$ -Protease inhibitor is also an inhibitor of certain activated coagulation factors. An assay, which may be useful in the diagnosis of protein-losing enteropathy in dogs, measures increased  $\alpha_1$ -protease inhibitor concentrations in feces.

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## 10.3.5 $\alpha_2$ -Macroglobulins

$\alpha_2$ -Macroglobulins are large (approximately 725 kd) glycoprotein protease inhibitors with broad specificity. Both  $\alpha_1$ - and  $\alpha_2$ -migrating macroglobulin protease inhibitors have been identified in dog plasma. These protease inhibitors are able to bind and clear proteolytic enzymes released from neutrophils during inflammation, pancreatic proteolytic enzymes, and certain activated coagulation factors. The serum  $\alpha$ -macroglobulin concentration is generally decreased in dogs with acute pancreatitis, because the  $\alpha$ -macroglobulin–protease complexes are removed from the circulation by macrophages and hepatocytes.

## 10.3.6 Antithrombin III

Antithrombin III inhibits several serine proteases; thrombin, factor Xa, and factor IXa are the major ones targeted. Both  $\alpha$ - and  $\beta$ -migrating isoforms of antithrombin III have been found in rabbits and humans. Antithrombin III requires the presence of glycosaminoglycans such as heparan sulfate (located on cell surfaces and in the extracellular matrix) or exogenous heparin for optimal activity. Plasma antithrombin III can be measured by using chromogen assays. The antithrombin III concentration may be low in plasma because of decreased production (severe liver disease), loss (protein-losing nephropathies and protein-losing enteropathies), or consumption in hypercoagulable states. The antithrombin III concentration is increased in cats with various disease conditions, suggesting that it behaves as an APP in this species.

## 10.3.7 C-Reactive Protein

C-reactive protein (CRP) was named for its ability to bind to the polysaccharide C fraction of pneumococci. In addition to binding to membranes of some infectious agents, CRP appears to promote complement activation and can bind to nuclear chromatin of damaged cells. This protein's molecular weight is about 100 to 155 kd, depending on species. Unlike horse and human CRPs, which are not glycoproteins, two of the five subunits of dog CRP are glycosylated. CRP migrates in the  $\gamma$  region on protein electrophoresis in dogs and humans and between the  $\beta$  and  $\gamma$  regions in horses. CRP is a major APP, increasing rapidly in the plasma of dogs, horses, and pigs in association with inflammation. It is not a significant APP in cats or ruminants.

## 10.3.8 Serum Amyloid A

Serum amyloid A (SAA) is a heterogenous low-molecular-weight protein (12 kd in humans) bound to high-density lipoproteins (HDLs) in all species studied. This nonglycosylated apolipoprotein is the precursor of amyloid A, which is the main fibrillar component of reactive amyloid deposited in tissues during secondary amyloidosis. The physiologic function of SAA is unknown. A protective role during inflammation has been hypothesized, because SAA is a major APP that increases rapidly in association with inflammation. The SAA concentration is also increased in Abyssinian cats with familial amyloidosis.

## 10.3.9 Ceruloplasmin

Ceruloplasmin is a glycoprotein with a molecular weight of 100 to 155 kd, depending on species. It contains most of the copper in the circulation in all species studied, except for dogs, in which ceruloplasmin accounts for only about 40% of the plasma copper content. Ceruloplasmin migrates in the  $\alpha_2$ -region in human serum but in the  $\alpha_1$ -region in horse serum on protein electrophoresis. In addition to being a copper transport protein,

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ceruloplasmin has ferroxidase enzyme activity that facilitates the oxidation of ferrous iron ( $\text{Fe}^{+2}$ ) to ferric iron ( $\text{Fe}^{+3}$ ), a process involved in iron mobilization from tissue stores. It also appears to function as an antioxidant in plasma. Ceruloplasmin is a mild to moderate APP that increases in concentration in association with inflammation.

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### 10.3.10 Transferrin

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Transferrin is the major iron-binding metalloprotein found in plasma. It has a molecular weight of about 80 kd and is a major component of the  $\beta_1$ -globulin band on electrophoresis. It is primarily responsible for iron transport throughout the body. The total iron-binding capacity (TIBC) of serum is a measure of serum transferrin concentration because insignificant amounts of circulating iron are bound to other proteins. Serum TIBC is low-normal or decreased in the anemia of inflammatory disease, because transferrin is a negative APP. Serum TIBC is increased in some species with iron deficiency (horses, cattle, pigs) but not in dogs.

### 10.3.11 Ferritin

Ferritin is present in much lower concentrations in plasma than is transferrin. Ferritin is generally not believed to contribute substantially to iron transport in plasma. However, plasma ferritin iron content is much higher in dogs than it is in humans, suggesting that ferritin plays a role in plasma iron transport in dogs. The serum ferritin concentration correlates with tissue iron stores in domestic animals. Consequently, the serum ferritin concentration can help differentiate true iron deficiency (serum ferritin is low) from the anemia of inflammatory disease (serum ferritin is normal or high), but commercial assay kits for ferritin are available only for humans. Ferritin is a positive APP; consequently, increased values in serum are expected in inflammatory conditions. Increased serum ferritin concentrations occur in chronic hemolytic anemia (e.g., pyruvate kinase deficiency) and in canine malignant histiocytosis.

### 10.3.12 Haptoglobin

Haptoglobin is a glycoprotein with a molecular weight of approximately 80 kd that contains approximately 20% carbohydrate. It exists in dimer and polymer forms and is a major component of the  $\alpha_2$ -protein band identified by electrophoresis in most species. Lysis of erythrocytes in the circulation (intravascular hemolysis) releases free hemoglobin into plasma, and hemoglobin tetramers spontaneously dissociate into  $\alpha$ - $\beta$  dimers that are bound by haptoglobin. Each haptoglobin monomer can irreversibly bind a hemoglobin  $\alpha$ - $\beta$  dimer, preventing some hemoglobin loss (and therefore iron loss) in the urine after intravascular hemolysis. Haptoglobin also assists in the protection against bacterial infections by binding to free hemoglobin in infected tissues, limiting iron availability for bacterial growth. In addition, haptoglobin functions as an antioxidant, because free hemoglobin promotes oxidative injury, which is inhibited by binding to haptoglobin. Finally, haptoglobin is reported to inhibit various aspects of neutrophil function.

The hemoglobin-haptoglobin complex is removed from plasma by hepatocytes and macrophages, and the presence of a low plasma haptoglobin concentration in dogs, cats, and horses suggests that recent or ongoing intravascular hemolysis is present. A low plasma haptoglobin concentration may also be present in animals with severe cirrhosis. The plasma haptoglobin concentration is usually very low or undetectable in healthy cattle and sheep; consequently, this assay cannot be used to seek evidence of intravascular hemolysis or cirrhosis in these species. Haptoglobin is an APP, and the measurement of increased concentrations in plasma provides evidence of inflammation in all domestic animals examined, although increased values can also result from glucocorticoid administration in some species (dogs and cattle). Only free haptoglobin is detected in

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spectrophotometrical haptoglobin assays; consequently, total plasma haptoglobin concentrations cannot be measured accurately in hemolyzed samples in which some or all of the haptoglobin is bound to hemoglobin.

## 10.3.13 Hormone-Binding Proteins

Hormone-binding proteins bind low-molecular-weight hormones, preventing the hormones from being rapidly filtered by the kidneys. These include corticosteroid-binding globulins, sex hormone-binding globulin, and thyroxine-binding globulin.

## 10.3.14 Fibrinogen

Fibrinogen has a molecular weight of 330 kd and contains three pairs of dissimilar polypeptide chains linked by disulfide bonds. It is a glycoprotein (approximately 3% to 5% carbohydrate) that migrates in the  $\beta$  region or between the  $\beta$  and  $\gamma$  regions on plasma protein electrophoresis. Fibrinogen is needed for normal platelet-to-platelet binding during platelet aggregation, and it is the precursor of fibrin in blood coagulation. Fibrin deposited in tissues provides scaffolding for inflammatory cells, fibroblasts, and endothelial cells. Plasma fibrinogen is an APP that increases most consistently in ruminants and horses with inflammatory diseases. The fibrinogen concentration can easily be estimated by measuring the total plasma protein concentration with a refractometer before and after heat precipitation of fibrinogen (see [Chapter 2](#)). Although high fibrinogen concentrations can be detected, this screening test underestimates the fibrinogen concentration, and therefore it is not sensitive enough to demonstrate low fibrinogen concentrations. Fibrinogen concentrations may be low, normal, or high in animals with disseminated intravascular coagulation. Although fibrinogen is consumed in the formation of thrombi, conditions that cause disseminated intravascular coagulation may also stimulate increased fibrinogen synthesis. The fibrinogen concentration is low in animals bitten by eastern diamondback rattlesnakes because their venom contains an enzyme called *crotalase* that degrades fibrinogen.

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## 10.3.15 Immunoglobulins

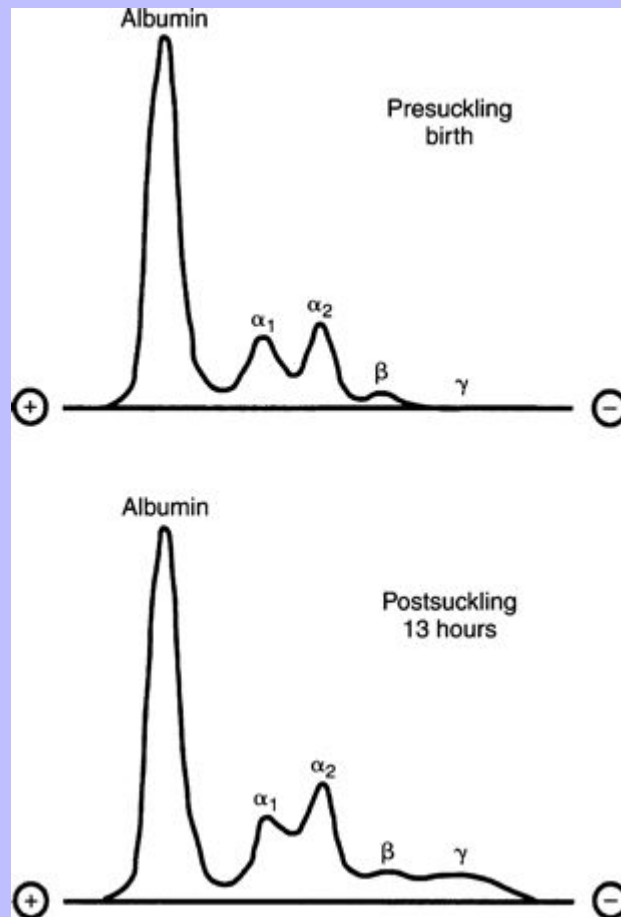
Immunoglobulin (Ig) proteins are produced by B lymphocytes and plasma cells. Immunoglobulins (or antibodies) are essential in the humoral immune responses to foreign antigens. Immunoglobulins are very heterogeneous, even within classes and subclasses. Immunoglobulins of the IgG class migrate in the  $\gamma$  region on electrophoresis, and IgM immunoglobulins migrate in the  $\beta$  region. IgA and IgE migrate in the  $\beta$ - $\gamma$  region. Of the total immunoglobulin present in serum of healthy dogs, approximately 85% is IgG, 10% is IgM, 5% is IgA, and less than 0.2% is IgE. IgD is not present in all species. IgD is present in small amounts in plasma of primates, rodents, and probably dogs but not in pigs, rabbits, or ruminants. Immunoglobulins are absorbed from colostrum during the first 24 hours after birth in domestic mammals, providing humoral immunity for the newborn animals until they can produce immunoglobulins on their own in sufficient amounts to provide protection against infectious agents.

## 10.4 CLINICAL APPLICATIONS OF SERUM PROTEIN ELECTROPHORESIS

Serum protein electrophoresis is usually performed when serum protein levels are increased, but it may be used as a screening test for immunoglobulin (primarily IgG) deficiency, because immunoglobulins account for all of the protein that migrates in the  $\gamma$  region and some of the protein that migrates in the  $\beta$  region of electrophoretic membranes or gels. The absence and presence of IgG are demonstrated in serum electrophoretograms from a foal before and after absorption of colostrum ([Fig. 9-3](#)).

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Fig. 9-3



Serum protein electrophoretograms for a foal before and after suckling. The serum IgG concentration was less than 200 mg/dL before suckling and greater than 800 mg/dL after suckling.

Serum protein electrophoresis can provide useful information for determining the cause of an increased total serum protein concentration. Except in animals with dehydration, an increased total serum protein concentration usually indicates increased concentrations of immunoglobulins. Substantial increases can occur in the concentrations of nonimmunoglobulin proteins migrating in the  $\alpha$  and  $\beta$  regions, but these increases are usually not of sufficient magnitude by themselves to result in a high total protein concentration. An increase in the  $\alpha_2$ -globulin concentration is often observed in animals with inflammatory conditions. Studies in dogs have shown that most of this  $\alpha_2$ -globulin increase can be attributed to an increase in the acute-phase reactant protein haptoglobin (see Fig. 9-2). The concentration of haptoglobin also increases in dogs after glucocorticoid administration; consequently, an increased  $\alpha_2$ -globulin concentration does not necessarily indicate the presence of inflammation, at least in dogs.

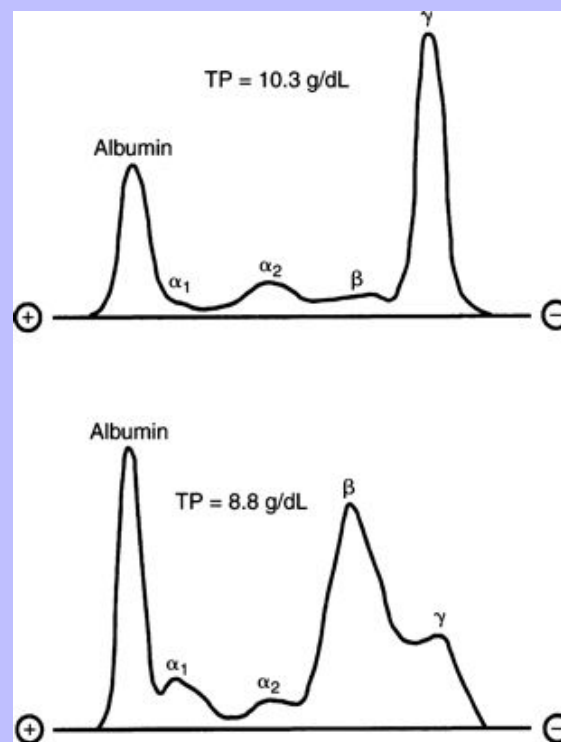
Increased serum immunoglobulin concentrations occur in either inflammatory or neoplastic conditions. When the increase appears as multiple peaks or a broad-based peak in the  $\beta$  and/or  $\gamma$  region of the electrophoretic tracing, the

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terms *polyclonal hyperglobulinemia* and *polyclonal gammopathy* are used to describe the increased immunoglobulin concentration (Fig. 9-4). Polyclonal hyperglobulinemia usually results from chronic inflammatory conditions, such as pyoderma, dirofilariasis, ehrlichiosis, and feline infectious peritonitis. As discussed previously, an increased  $\alpha_2$ -globulin concentration may also be present.

When the increased immunoglobulin concentration results from a single protein that appears as a sharp, narrow-based peak (similar to that seen for albumin) in the electrophoretic tracing, the terms *monoclonal hyperglobulinemia* and *monoclonal gammopathy* are used (see Fig. 9-4). Although it is a misnomer, the term *monoclonal gammopathy* is often used, even when the abnormal protein migrates in the  $\beta$  region of the electrophoretic membrane or gel. These abnormal proteins are sometimes referred to as *M proteins* or *paraproteins*. Most monoclonal gammopathies are produced by clonal proliferations of neoplastic cells of the B-lymphocyte series (multiple myeloma, plasmacytoma, primary or Waldenström's macroglobulinemia, lymphoma, and chronic lymphocytic leukemia). However, monoclonal gammopathies have also been reported, although rarely, in animals with amyloidosis and extensive nonneoplastic plasma cell proliferations (ehrlichiosis, leishmaniasis, and plasmacytic gastroenterocolitis in dogs and feline lymphoplasmacytic stomatitis in a cat) and also in the absence of an identifiable cause (idiopathic paraproteinemia or monoclonal gammopathy of undetermined significance). Biclonal gammopathies with two distinct peaks visible in electrophoretogram tracings have rarely been reported in animals with multiple myeloma or plasmacytoma (Fig. 9-5). These biclonal peaks may represent monomers and dimers of a single protein or may be two different proteins.

Fig. 9-4



Serum protein electrophoretograms from a cat with multiple myeloma and a monoclonal gammopathy (top graph), and a dog with chronic pyoderma and a polyclonal gammopathy (bottom graph).

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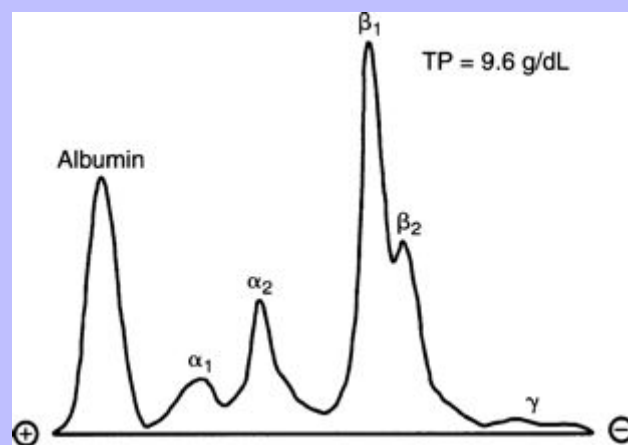
## 10.5 LIPOPROTEINS

Lipoproteins are synthesized in the intestinal mucosa and liver, and they transport water-insoluble lipids in the blood. Lipoproteins are high-molecular-weight water-miscible complexes composed of lipids (cholesterol, triglycerides, and phospholipids) and one or more proteins called *apolipoproteins*. Lipoproteins form pseudomicellar particles, in which the hydrophilic moieties of the phospholipids, apolipoproteins, and free cholesterol are arranged on the surface and the hydrophobic triglycerides and cholesterol esters are oriented toward the interior to form the core of these globular particles.

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Fig. 9-5



Serum protein electrophoretogram from a dog with multiple myeloma and a resultant biclonal IgA gammopathy. Additional studies indicated that two separate IgA proteins, which probably differed in light chain class, were present.

Apolipoproteins are required for the normal structure of lipoproteins, stabilizing aqueous micellar suspensions of polar and nonpolar lipids. Apolipoproteins are essential for the binding of lipoproteins to cellular receptors, and they function as cofactors for the lipolytic enzymes, lipoprotein lipase and lecithin/cholesterol acyltransferase.

Plasma lipoproteins vary in size, density, electrical charge, lipid and apolipoprotein composition, and metabolic function. Lipoproteins may be classified by density after ultracentrifugation or by electrophoretic mobility ([Table 9-2](#)). Lipoproteins listed in increasing order according to density, fractional protein content, and fractional phospholipid content but in decreasing order according to size and triglyceride content are chylomicrons, very-low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and HDLs.

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Table 9-2 Characterization of Lipoproteins in Serum from Dogs

Lipoprotein	Function	Size (nm)	Density (kg/L)	Mobility (electrophoresis)
Chylomicrons	Dietary lipids transport	75–1200	<0.960	Origin
VLDL	Hepatic triglyceride and cholesterol transport	30–80	<1.006	pre- $\beta$
LDL	Cholesterol transport to tissues	18–25	1.019–1.087	$\beta$
HDL <sub>1</sub>	Reverse cholesterol transport	10–35	1.025–1.100	$\alpha_2$
HDL <sub>2</sub>	Reverse cholesterol transport	9–12	1.063–1.100	$\alpha_1$

Values from Bauer JE: Comparative plasma lipid biochemistry and lipoprotein metabolism in dogs and cats. In Harvey JW, MacNeill A, Ramaiah S, Wamsley H. (eds): Proceedings of the 10 th Congress of the International Society of Animal Clinical Biochemistry. Gainesville, FL, International Society of Animal Clinical Biochemistry, 2002, pp 19–29.

HDL, High-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

## 10.5.1 Classes of Lipoproteins

### 10.5.1.1 Chylomicrons

Chylomicrons are very large lipoproteins of low density that remain at the origin when electrophoresis is performed. These lipoproteins are formed in the mucosal cells of the duodenum and jejunum and transport exogenous dietary triglycerides from the intestine to other tissues. Chylomicrons are secreted into lacteals and enter the circulation via the thoracic duct. Their core triglycerides are rapidly hydrolyzed by the action of lipoprotein lipase in capillary beds (primarily muscle and adipose tissue), where resultant fatty acids and glycerol are metabolized. Some phospholipid and apolipoprotein components of these triglyceride-depleted chylomicrons are transferred to HDLs, and the remaining chylomicron remnants are taken up by the liver. Chylomicrons have a short half-life in the circulation; consequently, chylomicrons are found in plasma in large amounts (chylomicronemia) after a meal containing fat, but chylomicrons are not normally measured in samples obtained after fasting.

### 10.5.1.2 Very-Low-Density Lipoproteins

VLDLs are primarily synthesized by the liver, although some intestinal formation can occur. VLDLs migrate in the pre- $\beta$  region on electrophoresis and transport the bulk of the endogenous triglycerides. Triglycerides in VLDLs are hydrolyzed by hepatic triglyceride lipase in addition to muscle and adipose tissue lipoprotein lipase. Residual VLDLs are transformed into LDLs.

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### 10.5.1.3 Low-Density Lipoproteins

LDLs are metabolic products of VLDLs. They migrate in the  $\beta$  region on electrophoresis. LDLs are primarily responsible for the transport of cholesterol into peripheral tissues. Cholesterol is needed as a component of cell membranes and for the formation of steroid hormones in organs, such as the adrenal cortex and ovaries.

### 10.5.1.4 High-Density Lipoproteins

HDLs are the smallest and most dense lipoproteins, migrating in the  $\alpha$  region on electrophoresis. HDL precursors are formed in the liver, and complete molecules are formed in the plasma by addition of remnants

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from other lipoproteins. HDLs transport cholesterol from tissues back to the liver in a process termed *reverse cholesterol transport*.

## 10.5.2 Species Differences in Lipoproteins

LDLs account for 50% or more of the lipoprotein content present in plasma of fasting humans, and cholesterol contained within LDLs is believed to contribute to the prevalence of atherosclerosis in humans. In contrast to humans, in most fasting domestic animals, HDLs account for about 50% to 75% of the lipoprotein content present in plasma, a feature that likely accounts for the rare occurrence of atherosclerosis in domestic animals. Most species have a single  $\alpha_1$ -migrating HDL band on electrophoresis, but about 50% of healthy dogs also have an  $\alpha_2$ -migrating HDL band, designated *HDL<sub>1</sub>*, and cats are reported to have three HDL fractions.

## 10.5.3 Disorders of Lipoprotein Metabolism

Disorders of lipoprotein metabolism may be either primary or secondary. Information concerning the lipoproteins involved may be obtained by visual examination of plasma. The appearance of turbidity or lactescence in centrifuged plasma or serum samples indicates the presence of hypertriglyceridemia associated with large amounts of chylomicrons and/or VLDLs in the samples. Chylomicronemia is indicated if a “cream” layer forms in serum or plasma allowed to stand undisturbed overnight in a refrigerator. Increased VLDLs are likely present if the underlying serum or plasma remains cloudy (with or without a cream layer) after overnight storage in the refrigerator.

Lipoprotein electrophoresis patterns alone may not provide definitive information concerning which lipoproteins are increased, because increased amounts of a lipoprotein class may result in a broad band on electrophoresis, and abnormal lipoproteins may have altered migrations on electrophoresis. For example, dogs with hypertriglyceridemia generally have increased  $\beta$ -migrating lipoproteins, which suggests increased LDLs, but this pattern in dogs may reflect increased VLDLs, LDLs, or both.

Chylomicronemia occurs in healthy animals after consumption of foods containing significant amounts of fat. Postprandial chylomicronemia can be avoided by fasting animals overnight before blood is collected. Secondary disorders of lipoprotein metabolism have been reported in dogs with high-fat diets ( $\alpha_2$ -migrating HDL<sub>1</sub> increase), pancreatitis, and/or diabetes mellitus (chylomicronemia and VLDL increase), hypothyroidism ( $\alpha_2$ -migrating HDL<sub>1</sub> increase and sometimes LDL increase), cholestasis ( $\alpha_2$ -migrating HDL<sub>1</sub> increase), and hyperadrenocorticism (VLDL and LDL increases). Hyperlipidemia has been reported in ponies with anorexia, primarily during the last trimester of pregnancy. Increased free fatty acids are released from adipose tissue in response to fasting. Excess free fatty acids are esterified to form triglycerides in the liver and are exported as VLDLs. Consequently, plasma VLDL levels are markedly increased in equine hyperlipidemia.

Primary idiopathic hyperlipidemia has been described in miniature schnauzers and beagles (chylomicronemia and/or VLDL increase). An inherited deficiency in lipoprotein lipase occurs in cats, resulting in chylomicronemia and/or increased VLDL levels. Primary hypercholesterolemia has been reported in cats with increased LDLs and Briard dogs with increased  $\alpha_2$ -migrating HDL<sub>1</sub>.

## 10.6 ADDITIONAL READING

M Andersson, E Sevelius: Abnormal microheterogeneity of haptoglobin in serum from dogs with various diseases. *Vet Rec.* **148**, 2001, 14–17.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- RJ Baker, VE Valli: Electrophoretic and immunoelectrophoretic analysis of feline serum proteins. *Can J Vet Res.* **52**, 1988, 308–314.
- JE Bauer: Comparative plasma lipid biochemistry and lipoprotein metabolism in dogs and cats. In Harvey, JW, MacNeill, A, Ramaiah, S, et al. (Eds.): *Proceedings of the 10th Congress of the International Society of Animal Clinical Biochemistry*. 2002, International Society of Animal Clinical Biochemistry, Gainesville, FL, 19–29.
- D Bienzle, DC Silverstein, K Chaffin: Multiple myeloma in cats: Variable presentation with different immunoglobulin isotypes in two cats. *Vet Pathol.* **37**, 2000, 364–369.
- FS Blaisdell, WJ Dodds: Evaluation of two microhematocrit methods for quantitating plasma fibrinogen. *J Am Vet Med Assoc.* **171**, 1977, 340–342.
- EB Breitschwerdt, BJ Woody, CA Zerbe, et al.: Monoclonal gammopathy associated with naturally occurring canine ehrlichiosis. *J Vet Intern Med.* **1**, 1987, 2–9.
- NR Brewer: Comparative metabolism of copper. *J Am Vet Med Assoc.* **190**, 1987, 654–658.
- MJ Burkhard, DJ Meyer, RA Rosychuk, et al.: Monoclonal gammopathy in a dog with chronic pyoderma. *J Vet Intern Med.* **9**, 1995, 357–360.
- JG Conner, PD Eckersall, J Ferguson, et al.: Acute phase response in the dog following surgical trauma. *Res Vet Sci.* **45**, 1988, 107–110.
- JG Conner, PD Eckersall, A Wiseman, et al.: Bovine acute phase response following turpentine injection. *Res Vet Sci.* **44**, 1988, 82–88.
- JJ Dascanio, CH Zhang, DF Antczak, et al.: Differentiation of chronic lymphocytic leukemia in the horse. A report of two cases. *J Vet Intern Med.* **6**, 1992, 225–229.
- SP DiBartola, JA Reiter, JB Cornacoff, et al.: Serum amyloid A protein concentration measured by radial immunodiffusion in Abyssinian and non-Abyssinian cats. *Am J Vet Res.* **50**, 1989, 1414–1417.
- KJ Diehl, MR Lappin, RL Jones, et al.: Monoclonal gammopathy in a dog with plasmacytic gastroenterocolitis. *J Am Vet Med Assoc.* **201**, 1992, 1233–1236.
- M Dorfman, DS Dimski: Paraproteinemias in small animal medicine. *Comp Contin Educ Pract Vet.* **14**, 1992, 621–632.
- FH Drazner: Multiple myeloma in the cat. *Comp Contin Educ Pract Vet.* **4**, 1982, 206–216.
- PD Eckersall: Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. *Rev Med Vet (Toulouse).* **151**, 2000, 577–584.
- PD Eckersall, PK Saini, C McComb: The acute phase response of acid soluble glycoprotein,  $\alpha_1$ -acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein, in the pig. *Vet Immunol Immunopathol.* **51**, 1996, 377–385.
- DF Edwards, JW Parker, JE Wilkinson, et al.: Plasma cell myeloma in the horse. A case report and literature review. *J Vet Intern Med.* **7**, 1993, 169–176.
- A Font: Consider leishmaniasis in differential for monoclonal gammopathies in dogs (letter). *J Am Vet Med Assoc.* **208**, 1996, 184.
- K Ganrot: Plasma protein response in experimental inflammation in the dog. *Res Exp Med (Berl).* **161**, 1973, 251–261.
- JW George: Methemalbumin: Reality and myth. *Vet Clin Pathol.* **17**, 1988, 43–46.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- JW George: The usefulness and limitations of hand-held refractometers in veterinary laboratory medicine: An historical and technical review. *Vet Clin Pathol.* **30**, 2001, 201–210.
- JM Giraudel, JP Pages, JF Guelfi: Monoclonal gammopathies in the dog: A retrospective study of 18 cases (1986-1999) and literature review. *J Am Anim Hosp Assoc.* **38**, 2002, 135–147.
- JW Harvey, JM Gaskin: Feline haptoglobin. *Am J Vet Res.* **39**, 1978, 549–553.
- JW Harvey, CL West: Prednisone-induced increases in serum alpha-2-globulin and haptoglobin concentrations in dogs. *Vet Pathol.* **24**, 1987, 90–92.
- S Hayashi, T Jinbo, K Iguchi, et al.: A comparison of the concentrations of C-reactive protein and alpha1-acid glycoprotein in the serum of young and adult dogs with acute inflammation. *Vet Res Commun.* **25**, 2001, 117–126.
- PM Heegaard, J Klausen, JP Nielsen, et al.: The porcine acute phase response to infection with *Actinobacillus pleuropneumoniae*. Haptoglobin, C-reactive protein, major acute phase protein and serum amyloid A protein are sensitive indicators of infection. *Comp Biochem Physiol B Biochem Mol Biol.* **119**, 1998, 365–373.
- M Hoenig, C Wilkins, JC Holson, et al.: Effects of obesity on lipid profiles in neutered male and female cats. *Am J Vet Res.* **64**, 2003, 299–303.
- Hultén, C, RM Tulamo, MM Suominen, et al.: A non-competitive chemiluminescence enzyme immunoassay for the equine acute phase protein serum amyloid A (SAA)—a clinically useful inflammatory marker in the horse. *Vet Immunol Immunopathol.* **68**, 1999, 267–281.
- N Ikenoue, Y Saitsu, M Shimoda, et al.: Disease-induced alterations in plasma drug-binding proteins and their influence on drug binding percentages in dogs. *Vet Q.* **22**, 2000, 43–49.
- T Kajikawa, A Furuta, T Onishi, et al.: Changes in concentrations of serum amyloid A protein,  $\alpha_1$ -acid glycoprotein, haptoglobin, and C-reactive protein in feline sera due to induced inflammation and surgery. *Vet Immunol Immunopathol.* **68**, 1999, 91–98.
- JE Kent, CA Roberts: Serum protein changes in four horses with monoclonal gammopathy. *Equine Vet J.* **22**, 1990, 373–376.
- M Kristiansen, JH Graversen, C Jacobsen, et al.: Identification of the haemoglobin scavenger receptor. *Nature.* **409**, 2001, 198–201.
- L Langlais-Burgess, JH Lumsden, A Mackin: Concurrent hypoadrenocorticism and hypoalbuminemia in dogs: A retrospective study. *J Am Anim Hosp Assoc.* **31**, 1995, 307–311.
- AE Larsen, JL Carpenter: Hepatic plasmacytoma and biclonal gammopathy in a cat. *J Am Vet Med Assoc.* **205**, 1994, 708–710.
- CE Leifer, RE Matus: Chronic lymphocytic leukemia in the dog: 22 cases (1974-1984). *J Am Vet Med Assoc.* **189**, 1986, 214–217.
- A Lose, MC Marca: Study of the effect of total serum protein and albumin concentrations on canine fructosamine concentration. *Can J Vet Res.* **63**, 1999, 138–141.
- KF Lyon: Feline lymphoplasmacytic stomatitis associated with monoclonal gammopathy and Bence-Jones proteinuria. *J Vet Dent.* **11**, 1994, 25–27.
- NS Mandel, DG Esplin: A retroperitoneal extramedullary plasmacytoma in a cat with a monoclonal gammopathy. *J Am Anim Hosp Assoc.* **30**, 1994, 603–608.
- M Melamed Frank, O Lache, BI Enav, et al.: Structure-function analysis of the antioxidant properties of haptoglobin. *Blood.* **98**, 2001, 3693–3698.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- T Melgarejo, DA Williams, EK Asem: Enzyme-linked immunosorbent assay for canine  $\alpha_1$ -protease inhibitor. *Am J Vet Res.* **59**, 1998, 127–130.
- EM Milne, DL Doxey, JE Kent, et al.: Acute phase proteins in grass sickness (equine dysautonomia). *Res Vet Sci.* **50**, 1991, 273–278.
- A Montaser, C Tetreault, M Linder: Comparison of copper binding components in dog serum with those in other species. *Proc Soc Exp Biol Med.* **200**, 1992, 321–329.
- M Okumura, T Fujinaga, K Yamashita, et al.: Isolation, characterization, and quantitative analysis of ceruloplasmin from horses. *Am J Vet Res.* **52**, 1991, 1979–1985.
- EP Peterson, AC Meining: Immunoglobulin A and immunoglobulin G biclonal gammopathy in a dog with multiple myeloma. *J Am Anim Hosp Assoc.* **33**, 1997, 45–47.
- SK Ramaiah, MA Seguin, HF Carwile, et al.: Biclonal gammopathy associated with immunoglobulin A in a dog with multiple myeloma. *Vet Clin Pathol.* **31**, 2002, 83–89.
- JB Rollins, TD Shultz, RH Fiser: Serial measurements of serum protein, glycoprotein, and lipoprotein fractions in normal and Venezuelan equine encephalomyelitis-vaccinated ponies and burros. *Am J Vet Res.* **33**, 1972, 323–327.
- J Rossbacher, L Wagner, MS Pasternack: Inhibitory effect of haptoglobin on granulocyte chemotaxis, phagocytosis and bactericidal activity. *Scand J Immunol.* **50**, 1999, 399–404.
- CG Ruaux, RB Atwell: Levels of total  $\alpha$ -macroglobulin and trypsin-like immunoreactivity are poor indicators of clinical severity in spontaneous canine acute pancreatitis. *Res Vet Sci.* **67**, 1999, 83–87.
- CG Ruaux, RP Lee, RB Atwell: Detection and measurement of canine  $\alpha$ -macroglobulins by enzyme immuno-assay. *Res Vet Sci.* **66**, 1999, 185–190.
- M Satoh, T Fujinaga, M Okumura, et al.: Sandwich enzyme-linked immunosorbent assay for quantitative measurement of serum amyloid A protein in horses. *Am J Vet Res.* **56**, 1995, 1286–1291.
- KA Selting, GK Ogilvie, SE Lana, et al.: Serum alpha 1-acid glycoprotein concentrations in healthy and tumor-bearing cats. *J Vet Intern Med.* **14**, 2000, 503–506.
- E Sevelius, M Andersson: Serum protein electrophoresis as a prognostic marker of chronic liver disease in dogs. *Vet Rec.* **137**, 1995, 663–667.
- JE Smith: Iron metabolism and its disorders. In Kaneko, JJ, Harvey, JW, Bruss, ML (Eds.): *Clinical biochemistry of domestic animals*. 5th ed., 1997, Academic Press, San Diego, 223–238.
- T Stokol, JM Tarrant, JM Scarlett: Overestimation of canine albumin concentration with the bromocresol green method in heparinized plasma samples. *Vet Clin Pathol.* **30**, 2001, 170–176.
- T Taira, T Fujinaga, K Tamura, et al.: Isolation and characterization of  $\alpha_1$ -acid glycoprotein from horses, and its evaluation as an acute-phase reactive protein in horses. *Am J Vet Res.* **53**, 1992, 961–965.
- M Takiguchi, T Fujinaga, M Naiki, et al.: Isolation, characterization, and quantitative analysis of C-reactive protein from horses. *Am J Vet Res.* **51**, 1990, 1215–1220.
- IR Tizard: In *Veterinary immunology. An introduction*. 6th ed., 2000, WB Saunders Co, Philadelphia.
- K Watanabe, N Muranishi, Y Murata, et al.: Biochemical properties of canine serum ferritin: Iron content and nonbinding to concanavalin A. *Biometals.* **13**, 2000, 319–324.
- Watson, TDG: Lipoprotein metabolism in dogs and cats. *Comp Haematol Int.* **6**, 1996, 17–23.
- Watson, TDG, S Love: Equine hyperlipidemia. *Comp Contin Educ Pract Vet.* **16**, 1994, 89–97.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

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EG Welles: Antithrombotic and fibrinolytic factors—a review. *Vet Clin North Am Small Anim Pract.* **26**, 1996, 1111–1127.

D Werling, F Sutter, M Arnold, et al.: Characterisation of the acute phase response of heifers to a prolonged low dose infusion of lipopolysaccharide. *Res Vet Sci.* **61**, 1996, 252–257.

MS Whitney: Evaluation of hyperlipidemias in dogs and cats. *Semin Vet Med Surg (Small Anim).* **7**, 1992, 292–300.

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## 11 Chapter 10 Hepatobiliary and Skeletal Muscle Enzymes and Liver Function Tests

*For the study of medical diseases of the liver, it is essential that the pathologist be apprised of the clinical findings and the results of laboratory tests and radiographic studies. The correct diagnosis is most likely to be reached by the pathologist and clinician working as a team.*

Kamal Ishak MD, PhD, eminent hepatic pathologist

### 11.1 MICROANATOMY OF THE LIVER

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The microanatomy of the liver is discussed to demonstrate the impact of hepatic disease on the development of clinical signs and liver test abnormalities and to facilitate the integration of the biopsy report findings with the clinicopathologic findings.

#### 11.1.1 Hepatic Functional Units (Fig. 10-1)

The concept of functional units of the liver is based on models of microcirculation. Arterio-sinusoidal branches may open in various ways into groups of sinusoids, which bathe the cords of hepatocytes with arterial, venous, or mixed blood flow. The two models more commonly discussed are the polyhedral or classical lobule and the acinus concept. Each hepatic functional unit is approximately 1 to 2 mm in diameter, and there may be as many as 100,000 of these units in the liver. Microcirculatory abnormalities can cause liver-related dysfunction, resulting in adverse systemic events. These include altered iron metabolism, hepatic excretory dysfunction, and hepatic synthetic dysfunction. Hepatic microvascular dysplasia, with or without extrahepatic portosystemic shunting, is a congenital disorder in dogs and can cause hepatic encephalopathy and laboratory test abnormalities that reflect an altered vascular structure-function relationship.

#### 11.1.2 Physioanatomy of the Liver Cells Comprising the Hepatic Functional Unit (Fig. 10-2, see also Figs. 11-1 and 12-13)

The liver is composed of parenchymal cells and nonparenchymal cells. Each milligram of human liver contains approximately 170,000 parenchymal cells and approximately 30,000 nonparenchymal cells. The hepatocytes represent the enzyme-rich parenchymal cells; and the nonparenchymal cells consist of biliary epithelial cells, Kupffer cells, liver-associated lymphocytes, hepatic stellate cells, and endothelial cells. Because of their intimate structural-functional relationship, pathophysiologic mechanisms common to many hepatic and extrahepatic diseases result in abnormal liver test results and histopathologic findings that often appear nondiscriminatory.

Approximately 60% of the liver is composed of parenchymal cells. The hepatocyte is a polyhedral epithelial cell with specialized membrane functions. The basolateral or sinusoidal region faces the sinusoid and the perisinusoidal space. It is covered by abundant microvilli to facilitate the absorption of numerous substances from the blood and the secretion of metabolic products. The cell functions include protein synthesis, glucose metabolism, heme degradation, lipid metabolism, bile acid production and metabolism, and xenobiotic metabolism. The latter function is coordinated through a complex enzyme system referred to as the *mixed function oxygenase system*, of which the cytochrome P-450 component is most important. The metabolic alteration of drug molecules is known as a *phase I reaction*. The oxidative reactions can generate metabolites

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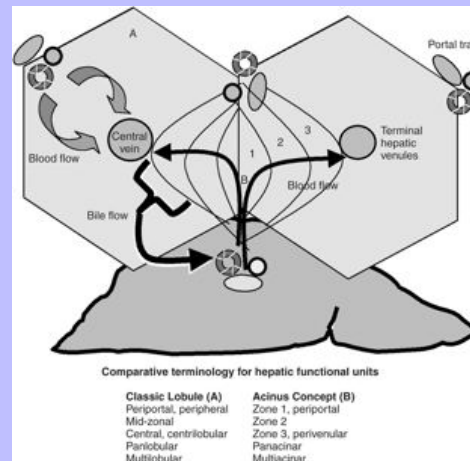
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that are more reactive than the parent compound, resulting in hepatic toxicity. Phase II biochemical reactions are handled by another superfamily of membrane-bound enzymes located in the hepatocyte, uridine diphosphate glucuronosyltransferases. They are involved in the elimination of xenobiotics and endogenous compounds including bilirubin, steroids, and thyroid hormone by means of glucuronidation. Glucuronide metabolites have increased aqueous solubility compared with the substrate, thereby enhancing their biliary and renal excretion. The canalicular region of the hepatocyte faces the intercellular space. The apposition of gutter-like hemi-canal of adjacent hepatocytes forms the bile canaliculus. The bile canaliculi drain into a progressively enlarging duct system formed by epithelial cells. Canaliculi and biliary epithelium express alkaline phosphatase and  $\gamma$ -glutamyl transferase (GGT) activity. The surface of the canalicular membrane is covered by microvilli and it contains microfilaments that have a contractile function to facilitate bile flow. A variety of extrahepatic infections release substances that cause dysfunction of the microfilaments, resulting in clinical jaundice referred to as *cholestasis of sepsis*.

Fig. 10-1

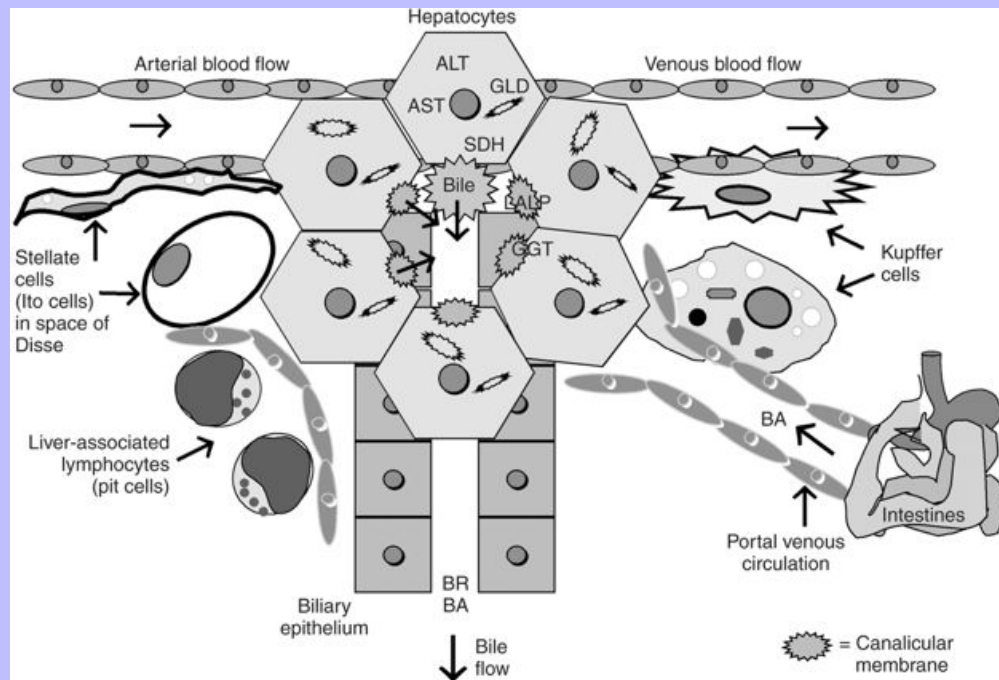


The classic lobule (A) represents the traditional concept of a hepatic histologic unit. The hexagonal lobule has a central vein in the center. With the exception of a few species with relatively abundant interlobular septa of connective tissue (pig, polar bear, raccoon, camel), it is difficult to discern microscopically. Portal tracts are located at the periphery of the lobule. Portal tracts usually contain one to two hepatic arteries, one portal vein, and one to two bile ducts. In human liver biopsy specimens, portal dyads (consisting of two of three of the aforementioned structures) are almost as common as portal triads in tissue located closer to the liver surface compared with deeper sites. Cords of hepatocytes contribute to the formation of the endothelium-lined sinusoids. A variable mix of arterial and portal blood percolates through the sinusoids en route to the central veins. The microscopically imperceptible plasma-filled spaces of Disse are sandwiched between the hepatocytes and richly fenestrated endothelial cells. Delicate terminal branches of a lymphatic plexus are laced about the terminal branches of the hepatic artery. The canalicular bile formed in the central area enters the terminal interlobular ductules and passes through a system of enlarging bile ducts en route to the portal tracts. The bile ductules surround the terminal hepatic portal venules in a spider web-like arrangement. The physioanatomic relationship, in collaboration with the biliary epithelial cholehepatic shunt pathway, facilitates the recycling of bile acids from bile to blood. Bile acids are the principal driving force of bile flow.

The simple acinus liver unit is defined as a variably sized grape-like cluster of hepatocytes with a portal tract coursing through its center when viewed three-dimensionally. The acinus lies between at least two terminal hepatic venules. Blood flows from the portal tract toward the terminal hepatic venules. A gradient of oxygen and other nutrients exists as a result of the vascular arrangement and the functional heterogeneity of the hepatocytes. Zone 3 hepatocytes are most susceptible to ischemic injury. A formation of at least three simple acini is referred to as a *complex acinus*, and a formation of three to four complex acini is referred to as an *acinar agglomerate*. In comparison, viewed from a two-dimensional perspective, the simple acinus occupies two adjacent classic lobules.

A side-by-side comparison of the terminology for the two hepatic structural-functional units is provided. The liver unit concept is helpful in relating the clinical pathologic findings to the location and extent of the hepatic disease. Because of the interdependent structural-functional relationships, similar abnormal hepatic test profiles are often associated with hepatic disease of diverse causes.

Fig. 10-2



The cells and their constituent enzymes that comprise the liver units described in Fig. 10-1 are illustrated. A functionally heterogeneous, polygonal hepatocyte population accounts for the majority of the liver cells. Each polygonal hepatocyte has a centrally placed nucleus surrounded by abundant granular, eosinophilic cytoplasm, which may have a foamy, rarefied appearance, depending on the amount of glycogen present. The hepatocytes may contain lipofuscin, the brown granular “wear and tear” pigment, or yellow-green-brown bile pigment after cholestasis. A variety of nonparenchymal cells make up approximately 30% to 40% of the liver cell mass. The biliary epithelium forms an enlarging duct system that collects bile formed by a group of canaliculi and transports it to the gut, recycling bile acids en route. Kupffer cells and liver-associated lymphocytes lie on the luminal aspect of the sinusoidal endothelial cells. The sinusoidal endothelial cells are microscopically inconspicuous, with only their flattened elongated nuclei protruding slightly into the lumen. The Kupffer cells belong to the mononuclear phagocytic system. Their irregular stellate shape, with protoplasmic processes extending through the larger endothelial fenestrae into the perisinusoidal space, is appreciated only with scanning electron microscopy. They appear elongated with light microscopy and may contain yellow-brown granular ceroid pigment after hepatocellular injury or golden-brown hemosiderin accentuated by Prussian blue staining in association with congenital portosystemic shunts and hemolytic anemia. The liver-associated lymphocytes represent an immunophenotypically heterogeneous resident population of natural killer, B, and T cells. They are scattered throughout the parenchyma with a prominence around the portal tracts in the human liver. The hepatic stellate cells—also referred to as *Ito cells*, *hepatic lipocytes*, *fat-storing cells*, *vitamin A*—storing cells, and *parasinusoidal cells*—are located in the spaces of Disse. They are inconspicuous with light microscopy but are easily seen scattered throughout the liver parenchyma when

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distended by a large prominent fat vacuole. They have a fundamental role in the development of hepatic fibrosis caused by injury.

The subcellular location of hepatobiliary enzymes is illustrated. ALT, SDH, and most of AST are located in the cytoplasm. GLD and a component of AST are associated with the mitochondria. An alteration in the integrity of the hepatocellular membrane with subsequent “leakage” of these enzymes causes their initial increase in the plasma. ALT activity is not high enough to be diagnostically useful in horses and ruminants. SDH and GLD are generally found in the liver of most domestic species. The bile canaliculi and biliary epithelium express ALP and GGT activity. An increase in their synthesis and release into the circulation is generally due to cholestatic liver disease or induction by endogenous cortisol (in dogs) or drugs.

*ALT*, Alanine aminotransferase; *AST*, aspartate aminotransferase; *GLD*, glutamate dehydrogenase; *SDH*, sorbitol dehydrogenase; *LALP*, liver isoenzyme of alkaline phosphatase; *GGT*,  $\gamma$ -glutamyl transferase; *BA*, bile acids; *BR*, total bilirubin.

Hepatocytes have a remarkable capability to regenerate. After partial hepatectomy in rats, the regenerative response is initiated within 24 hours with 80% regrowth within 5 days that is complete by the end of 3 weeks. The maximum regenerative response occurs within 72 hours after partial hepatectomy in dogs. The structure-function relationship is illustrated with the rat partial hepatectomy model. The circulating bile acid concentration increases approximately eightfold 6 hours after surgery. By postoperative day 1, it has decreased by 50%, another 50% by postoperative day 5, and it approximates the preoperative value 3 weeks after hepatectomy.

The biliary epithelium forms ducts of varying size that transport bile to the intestinal tract. Bile ducts modify canalicular bile. They reabsorb water and secrete bicarbonate-rich bile in response to stimulation by secretin, which is released from the duodenum. An anastomosing peribiliary vascular plexus derived from the hepatic artery drains into periportal sinusoids that supply the intrahepatic ducts. Conjugated bile acids are reabsorbed by the biliary epithelium by a bicarbonate-generating cholehepatic shunt pathway and recycled in collaboration with the peribiliary portal plexus. The recycling of conjugated bile acids is the principal physiologic process that impels the flow of bile (bile acid-dependent bile flow). Ursodeoxycholic acid amplifies bile flow through stimulation of the cholehepatic shunt pathway.

Kupffer cells are fixed tissue macrophages that are located in the lumen of hepatic sinusoids. These stellate to irregularly ovoid cells remove and degrade particulate and soluble material from the portal circulation and senescent erythrocytes, neoplastic cells, and immune complexes from the circulation. In addition to their normal role in host defense mechanisms, they play a prominent, underappreciated role in the pathogenesis of hepatic injury. Perturbation of Kupffer cells by endotoxin stimulates the release of tumor necrosis factor- $\alpha$ , a potent inducer of hepatocellular injury, and other proinflammatory cytokines such as interleukin-1, interleukin-6, interleukin-10, and interferon- $\gamma$ . In addition to these consequences of Kupffer cell-hepatocyte interactions, cytokine and growth factors are released after interactions between hepatocytes and endothelial cells or hepatic stellate cells. The response constitutes one mechanism for development of nonspecific reactive hepatitis and mildly abnormal liver enzyme levels associated with a variety of extrahepatic diseases. Hepatic microscopic alterations of varying severity often comprise portal lymphocytic infiltrates and bile ductular proliferation, small foci of single-cell necrosis with neutrophilic or lymphocytic inflammation, and granulomatoid reactions. The major impact of the disorder is the potential for unnecessary liver diagnostic tests, misdiagnosis as a primary liver disease, and/or distraction from identifying the underlying extrahepatic disease. Acute pancreatitis is a relatively frequent cause of reactive hepatitis in dogs and cats.

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Liver-associated lymphocytes are a heterogeneous resident population of lymphocytes in the liver. Immunophenotyping analysis demonstrates markers for B, T, and natural killer cells. The latter correspond to the liver's large granular lymphocytes or pit cells, so called because the electron-dense cytoplasmic granules resembled fruit pits on initial characterization. They are predominantly located around portal tracts where they interact with gut-derived antigens. The natural killer cells have numerous cytotoxic activities including lysis of tumor cells; production of multiple cytokines; and participation in immune responses against certain viruses, intracellular bacteria, and parasites. They are potent cytotoxic cells, comparable to lymphokine-activated killer cells, and in vitro studies show that the elimination of cancer cells occurs via the perforin/granzyme pathway with subsequent apoptosis. Although the precise functions of this resident lymphoid population in the liver are not clear, local immunologic reactions may be effected through the production of cytokines. It may be one reason that lymphocytes are a prominent inflammatory cell microscopically observed in the portal tracts in nonspecific reactive hepatitis.

The hepatic cell repertoire of a resident contingency of macrophages and a heterogeneous population of liver-associated lymphocytes permits an analogy to a lymphoid organ wherein the liver can react to antigenic stimuli. The antigens can be derived from extrahepatic inflammation and neoplasia and from the gut. Consequences of the reaction are abnormal liver enzyme levels and microscopic alterations referred to as *nonspecific reactive hepatitis*. Liver function test results are usually not abnormal.

Hepatic stellate cells—also referred to as *Ito cells*, *hepatic lipocytes*, *fat-storing cells*, and *parasinusoidal cells*—reside in the spaces of Disse. They are not readily visible with light microscopy because of their usual fusiform cell shape but become conspicuously obvious as plump fat-filled cells in a variety of conditions. Hypertrophy of hepatic stellate cells in humans occurs with vitamin A toxicity and in association with methotrexate or corticosteroid therapy. Similar associations have not been documented in animals. Hepatic stellate cell hypertrophy is a reported finding in dogs with hepatic nodular hyperplasia. The hepatic stellate cells have four notable functions that can contribute to hepatic pathophysiology and disease. They produce the extracellular matrix proteins in the normal liver and are the key players in the development of hepatic fibrosis when activated. They have contractile properties in response to nitric oxide and endothelin 1 and may have a role in the control of microvascular tone when activated in response to inflammation or injury. They express hepatocyte growth factor and may have a role in hepatic regeneration after hepatocellular necrosis. Hepatic stellate cells are a major site of vitamin A storage.

## 11.1.3 Liver and Skeletal Muscle Enzyme Tests

*Dr. Watson: "This is indeed a mystery. What do you imagine it means?"*

*Sherlock Holmes: "I have no data yet. It is a capital mistake to theorize before one has data. Insensible one begins to twist facts to suit theories, rather than theories to suit the facts."*

### A. Conan Doyle, Scandal in Bohemia

The circulating hepatic enzyme tests are grouped into those that indicate hepatocellular injury and repair and those that reflect increased production caused by cholestasis or drugs. The magnitude and duration of increase in the circulating enzyme activity are dependent on the enzyme's inherent tissue activity, subcellular location, rate of release, and rate of removal from the circulation and on the severity, duration, and type of disease. In general, the half-lives of enzymes in the circulation range from several hours to several days, and they are removed from the circulation by the macrophage system located in many organs.

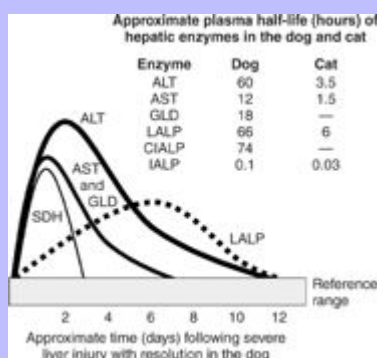
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## 11.2 LIVER ENZYMES INDICATIVE OF ALTERED PERMEABILITY OF THE HEPATOCYTE MEMBRANE (Fig. 10-3; See Fig. 10-2, Table 8-5) AND SKELETAL MUSCLE ENZYMES INDICATIVE OF ALTERED MEMBRANE PERMEABILITY (Fig. 10-4; See Fig. 13-6; Table 8-5)

### 11.2.1 Alanine Aminotransferase (EC 2.6.1.2; Alanine Transferase)

The aminotransferases, also termed *transaminases*, are enzymes that initiate the process of amino acid deamination. Amino groups are removed from amino acids and transferred to acceptor substances (transamination) for the formation of essential and nonessential amino acids, to be used for energy or to be further processed for storage as fat or glycogen. The transamination process is promoted by pyridoxine (vitamin B<sub>6</sub>). High alanine aminotransferase (ALT) activity is found in the cytoplasm of hepatocytes in dogs, cats, rodents, and primates. ALT activity in horses, ruminants, and marmosets is minimal. It is distributed throughout the liver lobule with the greatest concentration in the periportal area. Altered membrane permeability caused by injury, regenerative or reparative activity, or metabolic disturbance results in the release of ALT into the circulation. After acute injury, the magnitude of increase in the circulation roughly reflects the number of affected hepatocytes.

Fig. 10-3



The approximate magnitude and duration of increase of SDH, AST, GLD, ALT, and ALP levels in the circulation after severe hepatic injury with resolution in dogs are illustrated. The lack of complete resolution with weeks of duration is suggestive of persistent and/or progressive liver disease. Circulating enzyme levels would resolve sooner in cats compared with dogs. The circulating ALT level in horses and ruminants would not be notable. *ALT*, Alanine aminotransferase; *AST*, aspartate aminotransferase; *SDH*, sorbitol dehydrogenase; *GLD*, glutamate dehydrogenase; *LALP*, liver isoenzyme of alkaline phosphatase (ALP); *CIALP*, corticosteroid-induced ALP; *IALP*, intestinal ALP.

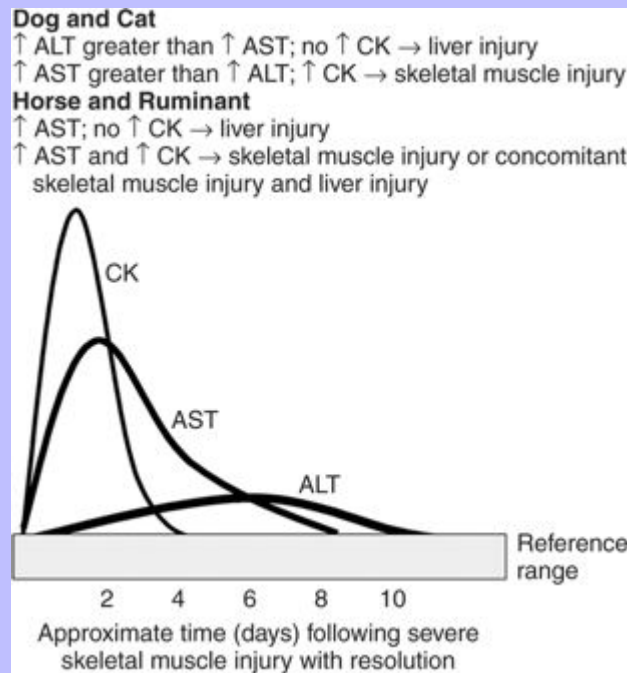
### 11.2.2 Aspartate Aminotransferase (EC 2.6.1.1; Aspartate Transaminase)

A variety of tissues have high aspartate aminotransferase (AST) activity. Striated muscle (skeletal, cardiac) and hepatocytes are noteworthy tissues. Determination of the circulating AST activity is used to detect hepatic injury in those species without high hepatic ALT activity. Striated muscle injury also causes an increase in

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circulating creatine kinase (CK) activity. Measurement of the circulating AST and CK activities in horses and ruminants is helpful in determining whether the increase in circulating AST is due to liver injury or skeletal muscle injury.

Fig. 10-4



The approximate magnitude and duration of increase in CK, AST, and ALT levels in the circulation after severe injury to the skeletal muscle in all domestic species are illustrated. In dogs and cats, the relative magnitude of ALT, AST, and CK levels help differentiate predominantly liver or skeletal muscle injury. In horses and ruminants, a rise in the AST level with or without a notable change in the CK level is compatible with liver injury. The AST and CK levels are increased in horses and ruminants with skeletal muscle injury alone or with concomitant liver injury. The latter is indicated by an increase in the sorbitol dehydrogenase or GLD levels. *ALT*, Alanine aminotransferase; *AST*, aspartate aminotransferase; *CK*, creatine kinase,.

After liver injury in dogs and cats, there is an increase in circulating ALT activity and, if it is severe, AST activity increases as well. The circulating AST activity will decrease more quickly than the circulating ALT activity. Sequential measurement will indicate whether/when the liver disease has resolved. A precipitous decrease may suggest inadequate parenchymal mass to support regeneration. Abnormal values that do not resolve indicate persistent disease. Both continued injury and reparation/regeneration probably contribute to the abnormal circulating levels.

Reduced circulating ALT and AST activities have been reported in humans and animals in association with phenothiazines and cefazolin. A low ALT value that was also inappropriate to the magnitude of the AST value in a dog was increased by the addition of pyridoxal-5'-phosphate, the bioactive metabolite of pyridoxine (vitamin B<sub>6</sub>), to the assay.

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## 11.2.3 Sorbitol Dehydrogenase (EC 1.1.1.14; L-Iditol Dehydrogenase), Glutamate Dehydrogenase (EC 1.4.1.3)

Hepatocytes are rich in sorbitol dehydrogenase (SDH) and glutamate dehydrogenase (GLD) in many species. SDH is located in the cytoplasm and GLD is associated with mitochondria. GLD is distributed throughout the liver lobule with the greatest concentration in the central area. SDH and GLD are particularly valuable for the detection of hepatocellular injury in those species that lack high ALT activity in the liver. GLD is routinely measured in clinical laboratories in some countries. In dogs the sensitivity of GLD for the detection of hepatic disease is similar to that of ALT.

## 11.2.4 Creatine Kinase (EC 2.7.3.2)

The cytoplasm of skeletal muscle cells has high levels of creatine kinase (CK), AST, and lactate dehydrogenase (LD) activity. CK catalyzes the reversible reaction of creatine phosphate in the presence of adenosine diphosphate (ADP) to form creatine and adenosine triphosphate (ATP), the energy source for muscle contraction. Creatinine is irreversibly formed from creatine by nonenzymatic dehydration. It rapidly diffuses into the circulation at a relatively constant rate proportionate to muscle mass. Creatinine is freely filtered by glomeruli and excreted in the urine.

The active form of CK is a dimer that includes a subunit (M or B) that affects the electrophoretic mobility, resulting in three isoenzymes—CK-1 (BB), CK-2 (MB), and CK-3 (MM). CK-1 is prominent in brain, CK-2 is primarily found in cardiac muscle, and CK-3 predominates in skeletal muscle and cardiac muscle. Increased CK activity in the circulation is generally indicative of skeletal muscle injury. The magnitude of the rise is dependent on the injury and tissue level. Cats have relatively less tissue CK than other species, and even a small increase above the reference range is notable. For unclear reasons, cats with anorexia can have increased circulating CK levels. The levels decrease several days after initiation of appropriate supportive alimentation. Metabolic diseases such as phosphofructokinase deficiency, hypothyroidism, hyperadrenocorticism, and malignant hyperthermia in dogs and muscular dystrophy in both dogs and cats can be associated with increased CK activity in the circulation. CK-1 can be measured in the cerebrospinal fluid as a nonspecific marker of central nervous system disease.

## 11.2.5 Lactate Dehydrogenase (EC 1.1.1.27)

Lactate dehydrogenase (LD) is a cytosolic enzyme that catalyzes the oxidation of L-lactate to pyruvate. It has high activity in many tissues. The enzyme is composed of two types of peptide chains, M and H, each under separate genetic control. Variable subunit composition of the two monomers results in five isoenzymes (LD1-5). Isoenzymes LD-1 and LD-2 are the prominent isoenzymes in erythrocytes, cardiac muscle, and kidneys; and LD-4 and LD-5 are located predominantly in liver and skeletal muscle. Because of the high tissue activity, relatively minimal tissue injury or hemolysis can result in high activity in the circulation.

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### 11.3 LIVER ENZYMES INDICATIVE OF IMPAIRED BILE FLOW OR DRUG INDUCTION (Fig. 10-5, See Figs. 10-2, 10-3, Table 8-5)

#### 11.3.1 Alkaline Phosphatase (EC 3.1.3.1) and $\gamma$ -Glutamyl Transferase (EC 2.3.2.2; $\gamma$ -Glutamyl Peptide: Amino Acid $\gamma$ -Glutamyl transferase)

Alkaline phosphatase (ALP) and  $\gamma$ -glutamyl transferase (GGT) show minimal activity in normal hepatic tissue but can become markedly increased in the plasma as a result of increased enzyme production stimulated by either impaired bile flow or drugs. The increased synthesis begins within hours, with subsequent appearance in the plasma caused by release mechanisms that are not clearly defined. These enzymes have a membrane location; ALP is associated with the canalicular membrane, and GGT is associated with epithelial cells that comprise the bile ductular system.

#### 11.3.2 Alkaline Phosphatase

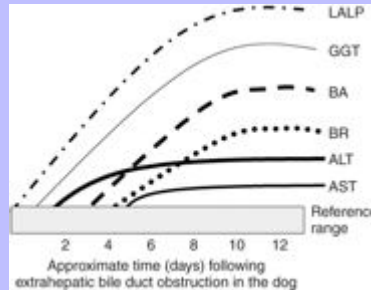
ALP activity is found primarily in liver, renal tubules, intestines, and bone (osteoblasts). ALP is a cell membrane-associated enzyme that catalyzes the alkaline hydrolysis of a large variety of substrates. The general metabolic function of this enzyme is poorly defined, but it is involved in the bone remodeling process (formation) and appears to be associated with lipid transport in the intestines. Circulating total ALP activity in healthy animals is composed of isoenzymes from the liver (LALP) and bone (BALP). The contribution of the bone isoenzyme to the total circulating activity is greatest in growing animals and can be increased in adult animals with bone cancer. The liver ALP activity increases the circulating total ALP activity in response to impaired bile flow (cholestatic hepatobiliary disease). Corticosteroid-induced ALP (CIALP) is an isoenzyme that is unique to dogs. As the name implies, it appears in the circulation of dogs in response to increased circulating glucocorticoid concentrations.

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Fig. 10-5



The approximate magnitude and sequence of increases in LALP, GGT, ALT, AST, BA, and BR levels after ligation of the common bile duct in dogs are illustrated. The magnitude of ALP increase is less in cats compared with dogs and less remarkable in horses and ruminants. Some bile acids have hydrophobic properties that act as potent detergents on lipid membranes. Their prolonged contact with the hepatocyte membrane alters its integrity, resulting in the release of cytosolic enzymes into the circulation. Retention of bile acids is linked to stimulating ALP synthesis and its release into the circulation. The majority of the increased total bilirubin level is composed of conjugated bilirubin. The increased level of the total conjugated bilirubin in the circulation generally consists of two forms—conjugated bilirubin loosely complexed with albumin by noncovalent binding and conjugated bilirubin irreversibly complexed with albumin by covalent binding referred to as *biliprotein* or *delta-bilirubin*. The contribution of each form to the increased total conjugated bilirubin level in the circulation is variable. *ALT*, Alanine aminotransferase; *AST*, aspartate aminotransferase; *LALP*, liver isoenzyme of alkaline phosphatase; *GGT*,  $\gamma$ -glutamyl transferase; *BA*, bile acids; *BR*, total bilirubin.

Different genes code the family of ALP isoenzymes. There is a tissue-nonspecific gene that codes for the isoenzyme in liver, kidney, and bone tissues. The isoenzyme produced in each tissue differs in the carbohydrate side chain and is referred to as an *isoform*. The intestinal ALP gene specifically codes for the formation of intestinal ALP. In humans, additional genes code for intestinal, placental, and placenta-like isoenzymes. The intestinal ALP gene also codes for the unique canine CIALP isoenzymes. The carbohydrate compositions of the two isoenzyme products of the intestinal ALP gene differ. The CIALP isoenzyme is glycosylated (addition of a sugar to a peptide or protein) with sialic acid. Previous investigations into the origin of the CIALP isoenzyme suggested that it might be an isoform of the intestinal ALP isoenzyme that was glycosylated in the liver. Subsequent work indicates that the hepatocyte has the ability to synthesize the CIALP isoenzyme in response to increased circulating glucocorticoid concentrations. It should be noted that the response of the canine liver to increased circulating glucocorticoid concentrations is complex. Levels of both the liver ALP isoenzyme and the CIALP isoenzyme rise in response to increased circulating glucocorticoid concentrations. The liver ALP isoenzyme level rises first in the circulation, followed by a rise in the CIALP isoenzyme level. The liver ALP isoenzyme is the predominant component of the total circulating ALP activity for at least 30 days, with a smaller contribution by the CIALP isoenzyme after about 1 week. The lag before the appearance of CIALP isoenzyme in the circulation may be related to delayed expression of a gene for CIALP synthesis in the liver.

Cholestasis, impaired flow of bile, is associated with changes in a variety of liver test results. The increased circulating levels are due to retention of bile constituents, enzyme leakage caused by altered hepatocyte membrane permeability resulting from the detergent effect of hydrophobic bile acids, or increased enzyme synthesis. A circulating half-life for ALP of 6 hours in cats limits its use as an early marker of cholestatic

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disease. In contrast, the dog liver has a robust ability to increase the production of ALP in response to the retention of bile and has a relatively long plasma half-life of 66 hours. There is minimal increase in circulating ALP after an acute hepatic injury. Within hours, the disease causes sufficient disruption of the hepatobiliary architecture to impair the flow of bile that signals the production of ALP by hepatocytes. During hepatic regeneration/repair, the circulating ALT activity decreases, whereas the circulating ALP activity may actually increase, at least in dogs, until de novo protein synthesis shuts down. The increase in the circulating ALP level associated with cholestasis in horses is intermediate between increases for dogs and cats and can be used diagnostically. Wide reference ranges limit the diagnostic utility of ALP in ruminants.	176 177
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A rise in circulating glucocorticoid levels, as previously mentioned, and anticonvulsant medications can increase circulating ALP activity in dogs. There is remarkable individual variation in the magnitude of these increases. It is noteworthy that there is no concomitant hyperbilirubinemia even when the circulating total ALP activity is dramatically increased by presence of the corticosteroid-induced ALP isoenzyme. (Refer to [Chapter 12](#) for further discussion of the use of the CIALP isoenzyme for the differential diagnosis of canine hyperadrenocorticism.) Drug-associated hepatitis can develop in association with the use of certain anticonvulsant medications. Features that are helpful in detecting its presence are a prominent rise in the circulating AST and/or ALT activities and/or a rise in the circulating GGT activity. These changes, especially in concert with an ill patient, suggest that a hepatic biopsy should be considered.

Levels of multiple circulating hepatic enzymes and bilirubin can be increased in association with feline hyperthyroidism; a rise in the circulating ALP activity is most common. A rise in the activity of isoenzymes from the liver, bone, and an unidentified source all contribute to its increase.

Circulating ALP activity has been shown to increase rapidly after a meal in humans and rats; the increase can be dramatic in the latter. The magnitude of increase is related to the lipid component of the meal. The mechanism for the increase appears to be related to the secretion of a particulate ALP onto the apical surface of the enterocyte, followed by release into both the lymphatics and the intestinal lumen. If this does occur in dogs and cats, the effect would not be noted clinically in fasted animals because of the short plasma half-life of intestinal ALP.

Reduced clearance of enzymes, especially ALP or GGT, resulting in increased circulating activity has been described in human medicine. It is possible that similar events may occur in veterinary medicine. Altered clearance in humans has been associated with viral infections, hepatic cirrhosis, diabetes mellitus, and chronic renal failure and has been observed in patients undergoing hemodialysis. An alteration of the terminal carbohydrate moiety (increased sialic acid content) probably alters receptor recognition for the removal of the enzyme. The rise in the circulating ALP activity in humans may persist for weeks after the resolution of a viral infection. In feline hepatic lipidosis, circulating ALP activity is dramatically increased. Increased production may be the sole explanation, but, because of the short half-life, reduced clearance of circulating ALP may be a contributing factor to the marked increase.

*Benign familial hyperphosphatasemia* refers to an inherited condition in human beings in which markedly increased circulating ALP activity is serendipitously identified in childhood. It persists into adulthood. The cause of the increased enzyme activity is not known, but it is not associated with underlying cholestatic disease, hence, the term *benign*. It is a biochemical curiosity with no adverse effect other than potentially causing an unnecessary evaluation for bone or hepatic disease. A biochemically similar condition has been described in Siberian husky pups.

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## 11.3.3 $\gamma$ -Glutamyl Transferase

GGT is a peptidase that is located on the cell membranes of a variety of tissues. It transfers the  $\gamma$ -glutamyl group from peptides and compounds that contain it to some acceptor. It is cleared from the circulation via the asialoglycoprotein receptor (galactose receptor) in the liver. A rise in circulating GGT activity is associated with cholestatic liver disease or an increase in circulating glucocorticoid levels in dogs. Anticonvulsant medications cause minimal to no increase in the circulating GGT activity in dogs, in contrast to the response in humans. Consequently, GGT may be a useful marker of chronic hepatitis caused by anticonvulsant medications. There is a discordant relationship between ALP and GGT in feline hepatic lipidosis. Even though both enzymes are indicators of cholestasis, circulating ALP activity is dramatically increased compared with circulating GGT activity. Circulating GGT activity is a useful marker of biliary tract disease in horses and ruminants in which the circulating ALP activity has limited diagnostic utility. The rise in the circulating GGT activity is due to increased synthesis stimulated by the retained bile.

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Bone has no GGT activity. In contrast to the increase that occurs in circulating ALP activity in association with growth and bone disease, circulating GGT activity remains unchanged. Colostrum and early breast milk have high GGT activity. Nursing neonates can have high circulating GGT activity. The rise in circulating GGT activity along with a rise in circulating bilirubin concentration may be particularly problematic in ill foals in which the biochemical changes mimic cholestatic disease. Measurement of the circulating GGT level can be used as a marker of passive immunoglobulin transfer. Renal tubular epithelial cells have relatively high GGT activity. Acute tubular injury results in a rapid increase in the GGT activity in the urine but not in the circulation. The measurement of urine GGT activity is a useful indicator of nephrotoxicity before changes in circulating urea nitrogen or creatinine develop.

Monitoring of the liver enzyme levels will determine whether and when resolution is complete. The rate of the decline in enzyme levels is usually more protracted than predicted by their plasma half-life because of the continued release and production of enzymes associated with the regeneration and reparation process. Clinical improvement often precedes the complete resolution of all liver test abnormalities. Lack of complete resolution suggests persistent primary liver disease or reactive hepatitis in extrahepatic disease.

## 11.3.4 Macroenzymes

In human patients, an unexplained, persistent increase in circulating enzyme activity can be due to the presence of a macroenzyme. The high-molecular-mass enzyme is either an immunoglobulin-bound or nonimmunoglobulin-bound type. The altered protein configuration impairs receptor recognition of the protein for its clearance. In humans, macroenzymes have been reported to cause increased circulating activities of ALT, AST, ALP, GGT, CK, LD, lipase, and amylase. An immunoglobulin-amylase complex (macroamylase) has been identified in dogs with increased circulating amylase activity and proteinuria but without clinical signs of pancreatitis. An increased circulating enzyme activity without clinical and/or histomorphologic abnormalities should prompt consideration of a macroenzyme as the cause.

## 11.4 TESTS DEPENDENT ON LIVER FUNCTION

*The multiple functions of the liver are exceeded in number only by the biochemical methods designed to test them.*

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Professor Dame Sheila Sherlock, MD, eminent hepatologist, 1918-2001

## 11.5 LIVER TESTS DEPENDENT ON SYNTHETIC FUNCTION OF THE LIVER

### 11.5.1 Albumin ([Fig. 10-6](#), [Box 10-1](#), see [Fig. 11-4](#); [Boxes 11-2](#), [16-1](#) and [16-2](#))

Albumin is exclusively synthesized by hepatocytes and traverses the lymph-filled spaces of Disse and the porous endothelium into the hepatic sinusoids for delivery into the systemic circulation. The principal function of albumin is to prevent plasma loss from capillaries by exerting colloid osmotic pressure. Hypoalbuminemia, a significant reduction in the circulating albumin level (generally to less than 1.5 g/dL), can result in edema and pleural and/or peritoneal effusions. A reduction of the albumin level in the circulation is generally due to increased loss or reduced synthesis. Increased loss is generally due to renal or intestinal disease. Reduced production is generally a consequence of liver insufficiency caused by significant loss of parenchymal mass or congenital portosystemic shunts or by down-regulation of albumin synthesis. Reduction of albumin synthesis can be prompted by hyperglobulinemia or by release of cytokines caused by extrahepatic inflammation. The rate at which hypoalbuminemia develops is dependent on the severity of inciting cause, the rate of hepatic synthesis, and the half-life of albumin.

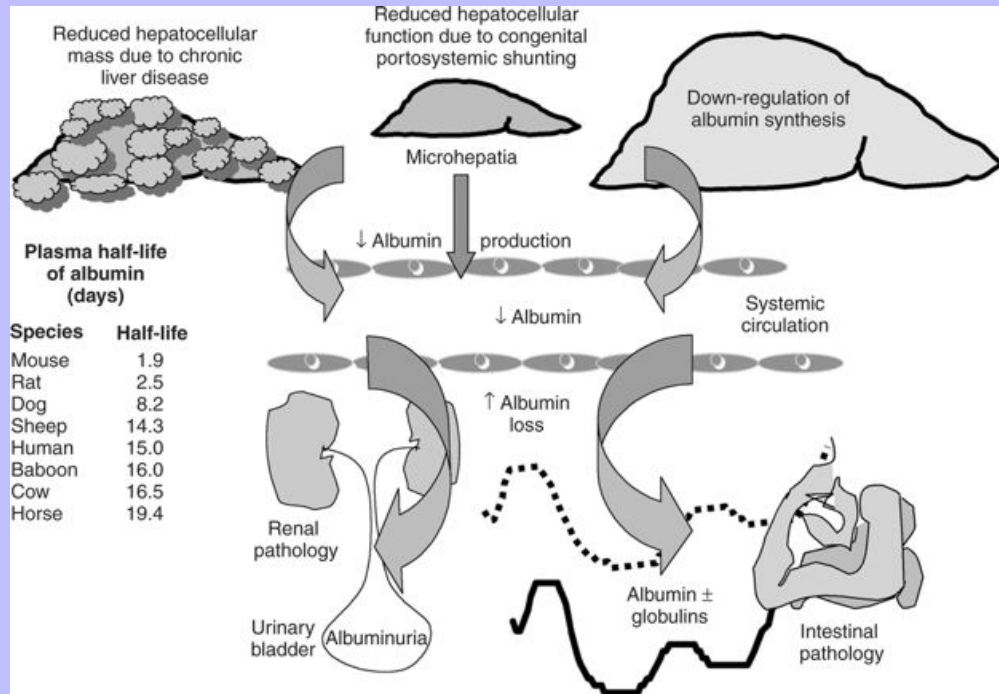
Because albumin and many of the globulins are produced in the liver, the protein content of hepatic lymph approximates 90% of the plasma total protein concentration. Increased hepatic venous pressure (e.g., as with right-sided heart failure) causes leakage of hepatic lymph into the abdomen and contributes to the formation of a protein-rich ascites. Distended capsular efferent lymphatics can be observed microscopically.

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Fig. 10-6



A reduction in the albumin level in the circulation is generally due to increased loss or reduced synthesis. Increased loss is generally due to renal or intestinal disease. Reduced production is generally a consequence of liver insufficiency caused by severe loss of parenchymal mass, congenital portosystemic shunts, or down-regulation of albumin synthesis. Reduction of albumin synthesis can be prompted by hyperglobulinemia or by release of cytokines caused by extrahepatic inflammation. The rate at which hypoalbuminemia develops is dependent on the severity of the inciting cause, the rate of hepatic synthesis, and the half-life of albumin. (From Kaneko JJ: Serum proteins and the dysproteinemias. In Kaneko JJ, Harvey JW, Bruss ML [eds]: Clinical biochemistry of domestic animals. San Diego, Academic Press, 1997, pp 117-138.)

## 11.5.2 Ammonia, Urea, and Uric Acid (Figs. 10-7 to 10-9) (Cases 10-1 to 10-5)

"... I am a great eater of beef, and I believe that does harm to my wit."

Sir Andrew Aguecheek, an alcoholic knight in Shakespeare's *Twelfth Night*, declaring self-recognition of meat intoxication

"...those who are mad on account of phlegm are quiet, but those on account of bile are vociferous, vicious and do not keep quiet."

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## Hippocrates of Cos (observations regarding the neuropsychiatric manifestations associated with either acute or chronic liver failure)

The level of ammonia in the circulation is derived from deamination of amino acids by the liver and from bacterial metabolism of gut content. The portal vein content of ammonia is approximately five- to ten-fold higher than the level in the circulation. The ammonia generated is metabolized to urea by the Krebs-Henseleit urea cycle in hepatocytes. Abnormal clearance caused by altered portal circulation, decreased metabolism caused by loss of hepatocellular mass, or congenital deficiency of a urea cycle enzyme leads to excessive ammonia in the circulation. Hyperammonemia can also occur in ruminants after the ingestion of large quantities of urea.

Hyperammonemia has been historically linked to cerebral dysfunction referred to as *hepatic encephalopathy (coma)*. Hepatic encephalopathy is a complex metabolic syndrome associated with marked liver disease or congenital abnormal portal circulation. Whether ammonia is a major contributor to its pathogenesis is debatable, but there is a reasonable correlation with the arterial ammonia concentration. The venous ammonia concentration is an unreliable biochemical marker of hepatic encephalopathy. The oral administration of ammonium chloride salt solution improved its use as a diagnostic test but increased the risk of inducing encephalopathy. Determination of the circulating bile acid level has largely replaced the clinical use of ammonium chloride. A reduction in the urea (blood urea nitrogen [BUN]) level in the circulation is an indirect indication of altered ammonia metabolism, since its formation is dependent on adequate hepatic metabolism of ammonia.

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### 11.5.2.1 BOX 10-1 Causes of Hypoalbuminemia

Increased loss

Protein-losing nephropathy (Case 10-1)

Protein-losing enteropathy\*

Hemorrhage\* (internal body cavity, tissue planes, or external-exterior gastrointestinal tract)

“Third space” sequestration (subcutaneous, body cavity)

Extensive skin lesion (burns)\*

Decreased production

Liver insufficiency

Congenital portosystemic shunts

Severe loss of hepatocyte mass

Down-regulation of synthesis

Cytokine-induced associated with extrahepatic sites of inflammation

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Hyperglobulinemia

Inadequate nutrition

Exocrine pancreatic insufficiency (maldigestion)

Intestinal disease causing malabsorption

Prolonged protein-restricted diet

Severe starvation

Hyperglobulinemia-induced down-regulation of synthesis

Chronic hepatic insufficiency

Prolonged reduced protein diet

Prolonged insufficient caloric intake

Maldigestion (exocrine pancreatic insufficiency)

Malabsorption

\* Globulins are often concurrently decreased.

The urea level may increase in the circulation as a result of gastrointestinal hemorrhage. The increase in gut-related ammonia formation generated by the degradation of the protein-rich blood is rapidly metabolized during the first pass of the portal blood through the liver. The result is an increase in the urea (BUN) level in the circulation without an appreciable change in the circulating creatinine concentration, unless there is a concomitant prerenal disorder that causes a reduction in the glomerular filtration rate.

Uric acid is derived from the catabolism of purine bases and circulates as ionized urate. In humans and nonhuman primates, its level in the circulation is regulated by the kidneys, but in most other mammals, it is eliminated by the liver. Hepatocytes oxidize uric acid to form water-soluble allantoin, which is renally excreted. A reduction in its metabolism in concert with a reduction in ammonia metabolism caused by portosystemic shunting can result in the formation of ammonium urate crystalluria with or without urolithiasis. Dalmatians are predisposed to ammonium urate crystalluria with urolithiasis because of a unique metabolic disturbance in the liver that causes incomplete oxidation of uric acid.

### 11.5.3 Coagulation Factors

All coagulation factors except endothelium-derived factor VIII are exclusively produced by hepatocytes. They synthesize other plasma proteins, such as plasmin and antithrombin III, which play key roles in the regulation of the coagulation system. Hepatic macrophages also remove fibrin degradation products from the circulation. Consequently, there is high risk for coagulation abnormalities caused by hepatobiliary disease. Measurements of prothrombin time (PT) and activated partial thromboplastin time (APTT) are reasonable screening tests for coagulation abnormalities. The synthesis of the active forms of factors II, VII, IX, and X is vitamin K–dependent. When there is insufficient vitamin K, the inactive forms of these coagulation factors can be detected

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in the circulation. They are referred to as *proteins induced by vitamin K absence/antagonists (PIVKA)*, and their measurement is another means of assessing the integrity of the coagulation system. Reduced flow of bile to the intestinal tract caused by intrahepatic or extrahepatic cholestatic disease can alter the absorption of vitamin K. Administration of vitamin K to the patient with jaundice before an invasive procedure is a prudent practice. The increase of bile constituents in the circulation as a result of cholestasis appears to have the potential to alter platelet function that can be assessed by determining the mucosal bleeding time. (See [Chapter 6](#) for a detailed discussion of hemostasis.)


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Fig. 10-7

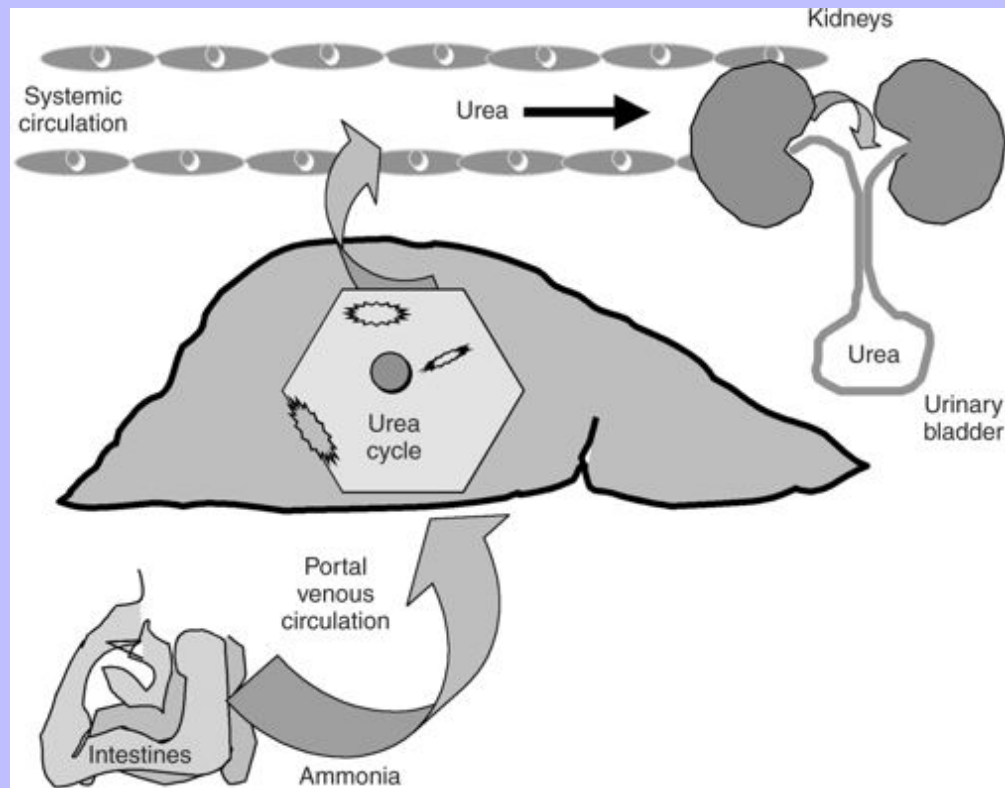
Comparison of mean age and selected laboratory values in dogs with hepatic microvascular dysplasia alone or in addition to extrahepatic portosystemic shunting

Variable	HMD <sup>†‡</sup>	HMD + PSS <sup>†‡</sup>
Age (y)*	3.0	1.4
MCV	73	60
FBA	41	159
PPBA	93	250
AST	59	113
ALP	78	265
Albumin	3.9	2.7
Glucose	107	87
Cholesterol	247	134
BUN	20	9.5
Creatinine	1	0.6



Liver insufficiency can adversely affect metabolic, synthetic, and excretory functions that have systemic consequences. Abnormal clinical pathology test results that reflect hepatic dysfunction are illustrated in dogs with hepatic microvascular dysplasia (HMD) alone or with extrahepatic portosystemic shunting (HMD + PSS). The frequency and magnitude of the abnormal laboratory findings are likely related to the degree of liver insufficiency. Abnormal iron metabolism adversely affects erythropoiesis, resulting in a reduced mean cell volume. Reduced synthetic ability is indicated by a reduction in albumin production and urea formation. Hepatic encephalopathy in some dogs is suggestive of hyperammonemia. An increase in the bile acid level in the circulation indicates altered integrity of the enterohepatic circulation. (From Allen L, Stobie D, Mauldin GN, et al: Clinicopathologic features of dogs with hepatic microvascular dysplasia with and without portosystemic shunts: 42 cases [1991-1996]. J Am Vet Med Assoc 1999;214:218-220.)

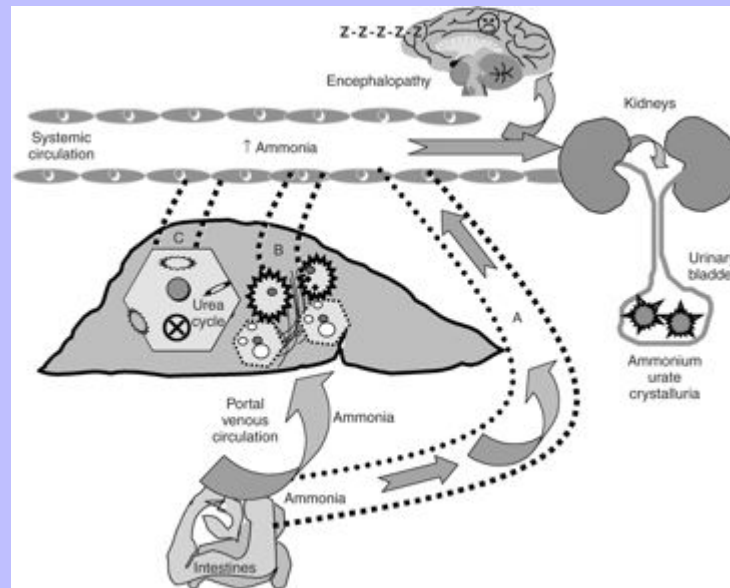
**Fig. 10-8**



Ammonia is formed from the hydrolysis of glutamine in the intestines and by the action of bacterial proteases, ureases, and amine oxidases on colonic contents. Ammonia is carried to the liver in the portal circulation where it is metabolized to urea by the Krebs-Henseleit urea cycle located in hepatocytes. Urea enters the systemic circulation and is filtered by glomeruli.

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Fig. 10-9



Hyperammonemia is generally caused by one of three disorders: congenital portosystemic shunts (A); severe loss of parenchymal mass caused by fulminant hepatic failure or chronic hepatitis, with or without acquired portosystemic shunts (B); or congenital deficiency of an enzyme comprising the urea cycle (C). Although congenital enzyme deficiency is probably a rare cause, it should be considered in a juvenile with encephalopathy, hyperammonemia, but total bile acids within reference range. The ingestion of large quantities of urea can result in hyperammonemia in ruminants. Ammonium urate crystalluria is most commonly associated with congenital portosystemic shunts. Hyperammonemia has been implicated as one of the causative factors in the development of encephalopathy. Dysfunction of the central nervous system has been associated with hepatic microvascular dysplasia with or without extrahepatic shunts (see Fig. 10-7).

11.5.4 **Glucose** (see Figs. 8-9, 12-13)

The liver modulates the glucose level in the circulation by glycogenesis and glycogenolysis, the formation and breakdown of glycogen, and gluconeogenesis, conversion of amino acids and glycerol into glucose. Generally, loss of hepatocellular mass is advanced before hypoglycemia develops subsequent to chronic liver disease. Hypoglycemia can develop as a result of acute diffuse hepatocellular necrosis. The presence of congenital microvascular disorders in the liver or portosystemic shunts can impair the ability of the liver to adequately regulate the glucose level in the circulation.

11.5.5 **Cholesterol and Lipoproteins** (see Figs. 12-15, 12-16)

The liver is the predominant organ involved in the synthesis of cholesterol, apolipoproteins, and all lipoproteins in the circulation, with the exception of chylomicrons. Despite the integral role of the liver in lipid metabolism and occurrence of dyslipidemia in liver disease, there are no tests that are clinically useful. A reduction in the

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circulating cholesterol level is associated with congenital portosystemic shunts. The cause of the reduction is not known but may be related to the altered bile acid metabolism. Acanthocytes are observed in association with chronic liver disease and congenital portosystemic shunts. Their formation is due to an abnormal lipid composition of the erythrocyte membrane that is likely an observable reflection of altered liver lipid metabolism. (See [Chapter 12](#) for additional discussion of lipid metabolism.)

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### 11.6 LIVER TESTS DEPENDENT ON EXCRETORY FUNCTION OF THE LIVER

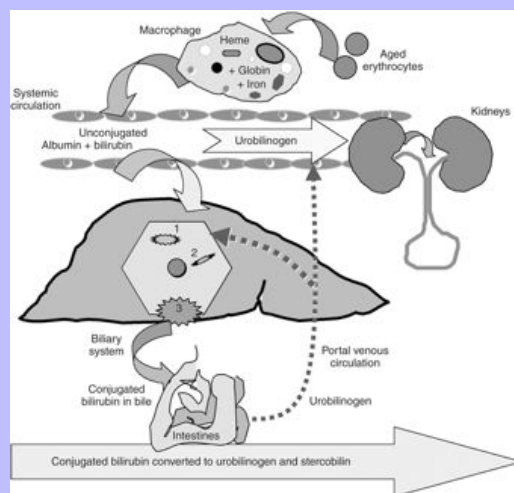
#### 11.6.1 Bilirubin ([Figs. 10-10, 10-11](#), see [Figs. 10-5, 11-1](#))

Unconjugated bilirubin is a pigmented compound produced largely from the degradation of heme from aged erythrocytes by the monocyte-macrophage system. A small proportion of unconjugated bilirubin is derived from hepatic cytochromes and ineffective erythropoiesis. Albumin transports the water-insoluble unconjugated bilirubin to the liver. Unconjugated bilirubin is also called *indirect-reacting bilirubin* because of the diazo reaction (van den Bergh test) used to differentiate it from conjugated bilirubin. The literature also refers to unconjugated bilirubin as *free bilirubin*, regardless of whether it is bound to albumin. On contract with the sinusoidal membrane of the hepatocyte, unconjugated bilirubin is dissociated from albumin, transported across the membrane, and bound to a cytoplasmic ligandin. Hepatocellular uptake is a very efficient process, resulting in a circulating unconjugated bilirubin half-life of minutes. The organic anion site for bile acid uptake is distinct from the one for bilirubin.

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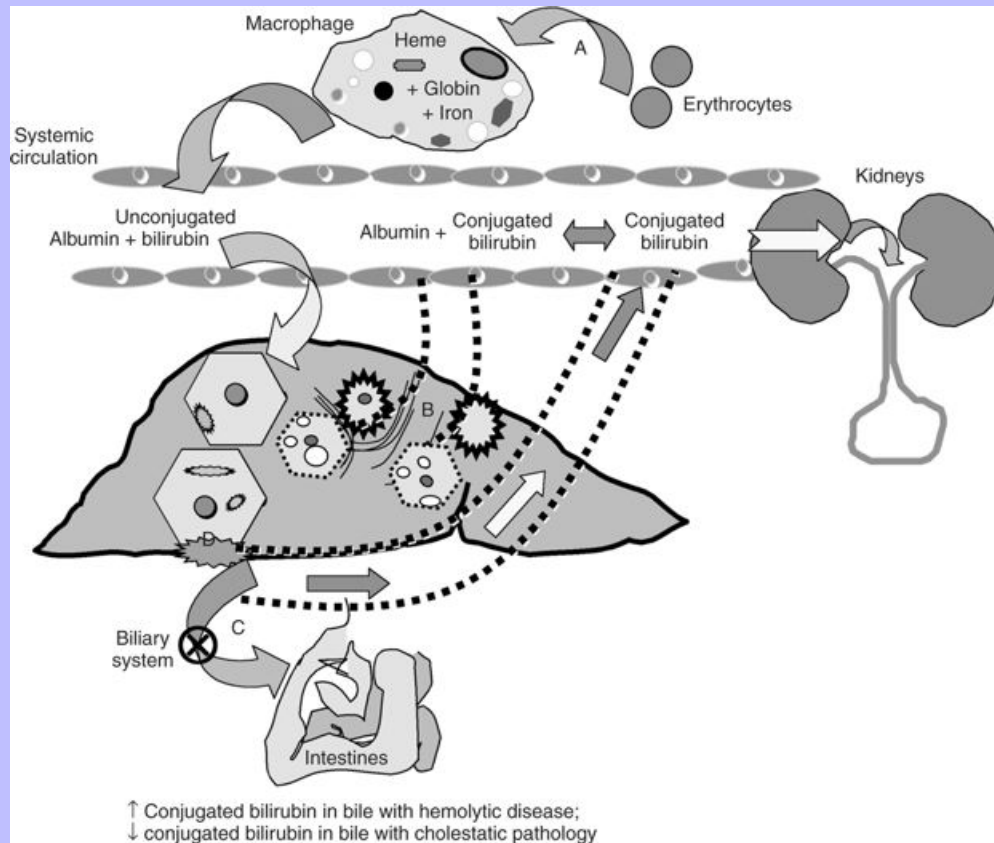
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Fig. 10-10



Macrophages remove aged erythrocytes and convert heme to unconjugated bilirubin bound to albumin for transport to the liver. Hepatocytes (1) take up the unconjugated bilirubin, (2) form conjugated bilirubin, and (3) excrete it via the canalicular membrane into the biliary system for transport in bile to the intestines. Intestinal bacteria convert conjugated bilirubin to urobilinogen and stercobilin. Some of the urobilinogen is absorbed into the portal venous circulation. Most of it is removed by the liver and excreted in bile. A small amount gains access to the peripheral circulation and is excreted in urine. Urobilinogen is oxidized to form urochrome, which imparts a yellow color. Oxidation of fecal stercobilin imparts a brown color.

Fig. 10-11



Hyperbilirubinemia is generally a consequence of hemolytic anemia (A) or cholestasis. Cholestasis can be due to intrahepatic disease (B), extrahepatic bile duct obstruction (C), or hepatocyte dysfunction (D), which is mediated by extrahepatic inflammation disorders in dogs and cats and inappetence in horses. When the level of conjugated bilirubin increases in the circulation, it is complexed with albumin by either noncovalent or covalent binding. Some of the conjugated bilirubin that is noncovalently bound to albumin can dissociate and appear in the urine. With resolution of cholestasis, the noncovalently bound form of conjugated bilirubin is rapidly removed from the circulation by the liver. The form of conjugated bilirubin that is covalently bound to albumin, biliprotein, is removed from the circulation concomitant with albumin degradation.

Bilirubin is conjugated with glucuronic acid to form water-soluble conjugated bilirubin, also called *direct-reacting bilirubin* because of the diazo reaction used for its determination. The metabolic process of glucuronidation is catalyzed by a member of the uridine diphosphate glucuronosyltransferase (UGT) family, UGT1A1. Various enzyme-inducing compounds of different inducing classes including phenobarbital, dexamethasone, clofibrate, and rifampicin stimulate induction of the microsomal enzyme. Canalicular excretion of conjugated bilirubin occurs against a concentration gradient that uses an ATP-dependent transport mechanism.

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Of the three hepatocyte-related steps involved the elimination of bilirubin—uptake, conjugation, and excretion—the process of excretion is rate-limiting. The complex canalicular excretory process is subject to adverse effects of constituents released from sites of extrahepatic bacterial infections. Inflammatory and infection-related substances can paralyze the canalicular membrane, resulting in a functional cholestasis referred to as *cholestasis of sepsis*. The hyperbilirubinemia can be dramatic with minimal histopathologic changes in the liver. The cholestasis spontaneously resolves when the extrahepatic disease is successfully managed. The canalicular transport of conjugated bilirubin is a carrier-mediated process competitively shared by cholecystographic dyes, sulfobromophthalein sodium (Bromsulphalein), and indocyanine green. The latter two dyes were commonly used to assess liver function.

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Conjugated bile acids are the principal constituents in bile that impel the flow of bile, referred to as *bile acid – dependent flow*. The bile carries conjugated bilirubin and excess cholesterol to the intestinal tract for excretion. Intestinal bacteria convert approximately one half of the bilirubin into urobilinogen, a highly soluble colorless compound. Some of the urobilinogen is reabsorbed by the intestines but most of it is promptly re-excreted by liver into the bile. The recycling of urobilinogen has no known function but is one example of the process termed *enterohepatic circulation*. The kidneys excrete the small amount of urobilinogen that enters the systemic circulation. On exposure of urine to air, the urobilinogen is oxidized to urobilin. Urochrome, a compound of urobilin, is the principal pigment in urine that imparts a yellow color. Oxidation of the fecal urobilinogen forms stercobilin, which imparts a brown color. The measurement of urinary urobilinogen with a reagent test strip is an archaic test that was used to differentiate hyperbilirubinemia caused by extrahepatic obstruction from intrahepatic cholestatic disease. It is not a reliable test because of the uncontrollable variables that affect its enterohepatic circulation. A rise in the circulating conjugated or unconjugated bilirubin level imparts a yellow color to tissues, referred to as *jaundice* or *icterus* when concentrations exceed approximately 2 mg/dL. The common causes of jaundice are accelerated destruction of erythrocytes, referred to as *hemolytic jaundice*, and hepatobiliary disease. The site of liver disease can be intrahepatic or extrahepatic. The predominant form of bilirubin in the circulation or the determination of the ratio of the conjugated bilirubin to unconjugated bilirubin was historically used for the differential diagnosis of jaundice in humans. Although the concept appears to be an attractive approach based on the kinetics of bilirubin metabolism, studies indicate that marked species variation in bilirubin metabolism precludes its diagnostic application in veterinary medicine. The magnitude of hyperbilirubinemia is not useful in the differential diagnosis of jaundice.

The conjugated bilirubin that increases in the circulation as a result of hepatobiliary disease is bound to albumin in one of two forms—one that is noncovalently bound and one that is covalently bound to albumin. The form that is noncovalently bound to albumin is easily dissociated. It has a plasma half-life of less than 24 hours. A small fraction that spontaneously dissociates from albumin is filtered by the kidneys and results in bilirubinuria. Bilirubinuria detected by either the diazo reagent pad of the dipstick or diazo reagent-impregnated tablet is an indication of impaired bilirubin excretion by the liver. Bilirubin is unstable when exposed to light. The test should be conducted soon after collection of the urine or stored in the refrigerator and tested within 24 hours.

Conjugated bilirubin can also become tightly bound to albumin by covalent binding. The conjugated bilirubin-protein complex is referred to as *biliprotein* or *delta-bilirubin* on the basis of its identification by high-performance liquid chromatography. Biliprotein has been demonstrated in the circulation of humans, rats, dogs, and cats that have had cholestatic liver disease. The amount formed is highly variable, ranging from negligible to most of the total bilirubin in the circulation. The irreversible binding necessitates that biliprotein be removed from the circulation concomitant with albumin degradation. The plasma half-lives of albumin in rats, cats, dogs, and humans are approximately 2, 6 (extrapolated value), 8, and 15 days, respectively. High levels of biliprotein cause prolonged hyperbilirubinemia and jaundice after resolution of the hepatobiliary disease. Most clinical chemistry analyzers cannot discriminate the two forms. Clinical chemistry analyzers that

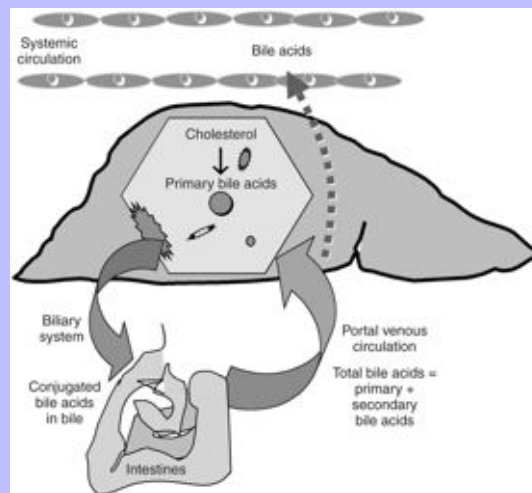
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use a dry chemistry reagent system can measure both forms that comprise the total amount of conjugated bilirubin in the circulation. Since the kidneys cannot filter biliprotein, it is possible to have relatively marked hyperbilirubinemia and jaundice with minimal bilirubinuria, depending on the amount of biliprotein formed.

Hemolytic jaundice can usually be identified by finding a marked reduction in the hematocrit. In ruminants, hemolytic anemia is a more common cause of jaundice than liver disease. Differentiating extrahepatic jaundice from intrahepatic cholestatic disease, as well as defining the type of intrahepatic disease, is problematic. Concomitant liver enzyme test profiles can be suggestive of hepatocellular injury or extrahepatic obstruction as the predominant disease resulting in jaundice, but these profiles are not diagnostically definitive. In general, the liver enzyme test profiles should be simply considered as indicators of liver disease. Additional testing is usually necessary to determine whether primary hepatobiliary disease or extrahepatic disease causing reactive hepatitis is responsible for the abnormal liver test results. Ultrasonography is valuable for assessing the hepatobiliary system for disease in dogs and cats. Often, examination of a biopsy specimen is necessary to further define any abnormal liver findings.

Horses manifest a relatively unique form of jaundice. The lack of food intake for more than 24 hours can result in hyperbilirubinemia. It appears that there is competition for or interference with hepatic uptake of unconjugated bilirubin by increased levels of free fatty acids or other metabolic constituents and/or reduced intrahepatic bile flow. The hyperbilirubinemia spontaneously resolves once the underlying cause of the inappetence is successfully managed. Measurements of the hematocrit and the circulating bile acid level, respectively, can be used to assess the other causes of jaundice, hemolytic disease, and hepatobiliary disease. A marked reduction of the former indicates hemolytic jaundice, and a marked rise in the latter is supportive of cholestatic liver disease.

Fig. 10-12



The primary bile acids are synthesized from cholesterol, conjugated to glycine or taurine, secreted into the canaliculi, and transported to the intestines by the biliary system. Intestinal bacteria metabolize some of the primary bile acids to form secondary bile acids. Conjugated bile acids are efficiently absorbed by the ileum into the portal venous circulation, transported to the liver, efficiently taken up by hepatocytes, and re-excreted into bile. The integrity of the enterohepatic circulation for bile acids results in a very effective recycling process.

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Lipemia can cause an erroneously high bilirubin value. A false-positive value should be considered if a high value is detected in a patient without jaundice.

### 11.6.2 Bile Acids (Figs. 10-12, 10-13, see Figs. 10-5, 10-7, 11-3)

The determination of the total bile acid level in the circulation as a test of liver function is based on a remarkably efficient recycling process referred to as *enterohepatic circulation*. The hepatobiliary system, terminal ileum, and portal venous circulation are the key components involved in the recycling of bile acids. Hepatobiliary disease and abnormalities of the portal venous circulation are the most common causes of increased bile acid levels in the circulation.

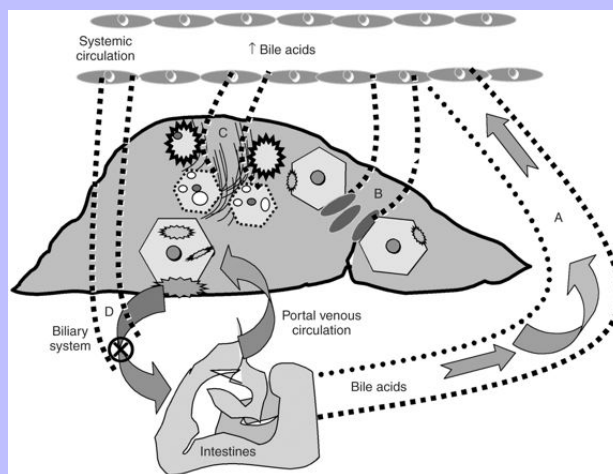
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### 11.6.3 Bile Acid Metabolism

Hepatocytes synthesize two primary bile acids from cholesterol, cholic acid and chenodeoxycholic acid, and conjugate them to glycine or taurine. They are secreted by the canalicular membrane into canaliculi for transport to the intestinal tract by the biliary system. Conjugated bile acids are efficiently absorbed (~95%) by the ileum into the portal circulation and transported to the liver for efficient first-pass uptake (~60% to 80%) by hepatocytes primarily located in zone 1. The transport systems for their uptake are distinct from those involved in bilirubin uptake. Conjugated bile acids are re-excreted into the biliary system for another enterohepatic journey during which they contribute the osmotic force that impels bile flow and provide surface-active detergent molecules that facilitate the process of solubilizing lipids for intestinal absorption. The small quantities of primary bile acids not reabsorbed in the ileum are dehydroxylated by anaerobic bacteria in the colon to form secondary bile acids. Some of these are absorbed into the portal venous circulation and recycled as well. Conjugated primary and secondary bile acids comprise the majority of the bile acid pool in the portal circulation.

Fig. 10-13



The increase in the bile acid level in the circulation is generally caused by one of four disorders: congenital portosystemic shunting (A), hepatic microvascular dysplasia (B), intrahepatic cholestatic disease (C), or extrahepatic bile duct obstruction (D).

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In dogs and cats, most of the bile formed is generally stored in the gallbladder before a meal. Cholecystokinin prompts gallbladder contraction after its food-stimulated release from the intestinal wall. Determinations of the bile acid level in the circulation before and approximately 2 hours after food ingestion, respectively, represent the fasting bile acid (FBA) and postprandial bile acid (PPBA) levels in the circulation. There appears to be individual physiologic variability for both the quantity of bile stored and degree of food-stimulated gallbladder contraction. It is likely that the food-stimulated cholecystokinin–gallbladder contraction relationship is not always physiologically robust in some ill animals. When the circulating bile acid concentration is within or near reference range values, it is possible that this physiologic variability results in a PPBA value that is similar to or less than the FBA value. Bacterial overgrowth in the small intestine in dogs may be another reason for such a finding. The circulating bile acid level in a random sample is determined in horses and ruminants.

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11.6.4

## Abnormalities of Bile Acid Metabolism \*

Violations of the integrity of the enterohepatic circulation that cause the bile acid level to rise in the systemic circulation are congenital abnormalities of the portal venous circulation, intrahepatic disease with or without acquired portosystemic shunts, and extrahepatic bile duct obstruction.

Congenital abnormalities of the portal venous circulation are often extrahepatic portosystemic shunts in most species. Clinical signs of hepatic encephalopathy and laboratory abnormalities detected by screening tests often prompt the measurement of the circulating bile acid level. The measurement of the PPBA often enhances the detection of the disorder in dogs and cats. In dogs, an abnormality of the microcirculation in the liver, referred to as *hepatic microvascular dysplasia*, can cause hepatic encephalopathy with changes in the laboratory test results that are more subtle than the clinicopathologic changes associated with concurrent extrahepatic portosystemic shunts.

Intrahepatic disease is often initially detected by abnormal liver enzyme test results. Abnormal liver test results and histologic changes can be due to primary liver disease or, more commonly, endocrine disorders or inflammatory disease in other organs that secondarily cause reactive hepatitis. Primary liver disease can be due to neoplasia or inflammatory changes that cause clinical disease or age-related hepatic alterations. In aged dogs, hepatocellular nodular hyperplasia is a common cause of abnormal liver enzyme test results and histopathologic findings of hepatitis with or without mild fibrosis, extramedullary hematopoiesis, hepatic stellate cell prominence, foci of lipid- or pigment-filled macrophages, and diffuse vacuolation or cytoplasmic rarefaction of hepatocytes without usually causing clinical signs or changes in liver function test results. In aged cats, lymphocytic/lymphoplasmacytic portal hepatitis appears to be a frequent microscopic finding in association with abnormal liver test results and anorexia and weight loss.

The measurement of FBA and/or PPBA can be helpful in determining whether to perform a liver biopsy. FBA or PPBA values greater than 25  $\mu\text{mol/L}$  for dogs and 15  $\mu\text{mol/L}$  for cats are generally associated with histopathologic changes in an adequate liver biopsy specimen. Since the circulating bile acid level increases before a rise in the bilirubin in the circulation caused by cholestatic disease, the measurement of the bile acid concentration in the circulation generally adds no useful diagnostic information in dogs and cats. In horses with hyperbilirubinemia, a circulating bile acid level within or near reference range values would suggest inappetence, rather than cholestatic liver disease, as causative.

A rise of the bile acids in the circulation secondary to liver disease or portosystemic shunting is associated with an increase in their urinary excretion. The measurement of bile acids in a random urine sample, expressed as ratio to creatinine, may detect liver disease in dogs and cats nearly as well as the serum bile acid test.

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\* The comments pertaining to bile acid metabolism are generally applicable to most domestic animals but have been severely truncated to focus on the use of measurement of bile acid levels as a liver function test. Bile acid metabolism is complex, and there is species variation in the predominant amino acid used for conjugation (e.g., the cat is an exclusive taurine conjugator), the type and quantity of each primary and secondary bile acid formed (e.g., hyocholic acid is a primary bile acid in swine), the efficiency of the enterohepatic circulation (e.g., ruminants have wide, variable reference ranges), and anatomic differences (e.g., the horse lacks a gallbladder). The appropriate literature is recommended for species-related physiologic nuances.

## 11.6.5 Miscellaneous: Dyes and Xenobiotics Dependent on Metabolism, Excretion, or Both

Sulfobromophthalein sodium (Bromsulphalein) and indocyanine green are two dyes of historical note that were used to assess liver function. The dye was administered by intravenous injection and its clearance time from the systemic circulation was determined. With the development of better strategies for assessing abnormal liver enzyme test results, neither dye clearance test has clinical utility. Clearance tests in which aminopyrine, caffeine, or lidocaine is used to assess functioning liver mass may have research applications. Carbon-labeled aminopyrine is demethylated by the hepatic microsomal enzyme system to form labeled carbon dioxide, which can be measured in the breath. Caffeine is a trimethylxanthine that is metabolized by the hepatic mixed-function oxidase system. The hepatic clearance capacity can be determined by monitoring its rate of elimination from the peripheral circulation. Lidocaine is an amino ethylamine that is metabolized by the hepatic cytochrome P-450 biotransformation system to form monoethylglycinexylidide (MEGX). Its rate of appearance in the peripheral circulation can be determined.

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## 11.7 ASSESSMENT OF ABNORMAL LIVER TEST RESULTS

Abnormal liver enzyme test results are indicators of liver disease or a response by hepatocytes to stimulation by corticosteroids or other enzyme-inducing drugs. Hepatobiliary disease may be due to primary liver disease or may be a reaction to inflammation in an extrahepatic organ. The magnitude and time course of liver enzyme test changes can be suggestive of acute or chronic disease or predominantly hepatocellular or cholestatic disease but seldom define a specific diagnosis. A diagnostic approach to define the cause of abnormal liver enzyme test results may include an assessment of breed predilection to liver disease or congenital portosystemic shunts, a history of concurrent treatments, a search for extrahepatic disease, measurement of the circulating bile acid level, imaging studies, and microscopic examination of a liver specimen. The primary objective of the microscopic examination of liver tissue is to translate morphologic abnormalities into clinically relevant terms useful in patient management. The hepatic pathology report should provide an overall assessment of lobular architecture; indicate whether neoplasia, copper accumulation, inflammation, fibrosis, or degenerative changes are present; and indicate the severity and extent of microscopic alterations. A specific diagnosis is often possible with neoplasia, feline hepatic lipidosis, and copper toxicosis but unlikely with inflammatory disease.

## 11.8 ADDITIONAL READING\*

\* New references added to the third edition.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- \*L Allen, D Stobie, GN Mauldin, et al.: Clinicopathologic features of dogs with hepatic microvascular dysplasia with and without portosystemic shunts: 42 cases (1991-1996). *J Am Vet Med Assoc.* **214**, 1999, 218–220.
- \*M Aminlari, T Vaseghi, MJ Sajedianfard, et al.: Changes in arginase, aminotransferases and rhodanese in sera of domestic animals with experimentally induced liver necrosis. *J Comp Pathol.* **110**, 1994, 1–9.
- \*S Asano, A Okano, K Ozawa, et al.: In vivo effects of recombinant human interleukin-6 in primates: stimulated production of platelets. *Blood.* **8**, 1990, 1602–1605.
- \*CE Balkman, SA Center, JF Randolph, et al.: Evaluation of urine sulfated and nonsulfated bile acids as a diagnostic test for liver disease in dogs. *J Am Vet Med Assoc.* **222**, 2003, 1368–1375.
- PE Ballmer, K Ballmer-Hofer, F Repond, et al.: Acute suppression of albumin synthesis in systemic inflammatory disease: An individually graded response. *J Histochem Cytochem.* **40**, 1992, 201–206.
- \*JE Bauer: Comparative lipid and lipoprotein metabolism. *Vet Clin Pathol.* **25**, 1996, 49–56.
- U Baumgartner, M Sellinger, G Ruf, et al.: Change of zone bile acid processing after partial hepatectomy in the rat. *J Hepatol.* **22**, 1995, 474–480.
- \*WA Beresford, JM Henninger: A tabular comparative histology of the liver. *Arch Histol Jpn.* **49**, 1986, 267–281.
- \*JR Bergman: Nodular hyperplasia in the liver of the dog: An association with changes in the Ito cell population. *Vet Pathol.* **22**, 1985, 427–438.
- TR Billiar, RD Curran, DJ Stuehr, et al.: An L-arginine-dependent mechanism mediates Kupffer cell inhibition of hepatocyte protein synthesis in vitro. *J Exp Med.* **169**, 1989, 1467–1472.
- \*P Bioulac-Sage, J Kuiper, Van Berkel, TJC, et al.: Lymphocyte and macrophage populations in the liver. *Hepatogastroenterology.* **43**, 1996, 4–14.
- \*CA Brady, CM Otto, TJ Van Winkle, et al.: Severe sepsis in cats: 29 cases (1986-1998). *J Am Vet Med Assoc.* **217**, 2000, 531–535.
- \*BA Breuhaus: Thyroid-stimulating hormone in adult euthyroid and hypothyroid horses. *J Vet Intern Med.* **16**, 2002, 109–115.
- \*C Briani, M Zaninotto, M Forni, et al.: Macroeozymes: Too often overlooked. *J Hepatol.* **38**, 2003, 119.
- \*SE Bunch, SE Johnson, JM Cullen: Idiopathic noncirrhotic portal hypertension in dogs: 33 dogs (1982-1998). *J Am Vet Med Assoc.* **218**, 2001, 392–399.
- \*AD Burt: Pathobiology of hepatic stellate cells. *J Gastroenterol.* **34**, 1999, 299–304.
- NR Butcher: Experimental aspect of hepatic regeneration. *N Engl J Med.* **277**, 1967, 686–696.
- \*SA Center, JF Randolph, T ManWarren, et al.: Effect of colostrum ingestion on gamma-glutamyltransferase and alkaline phosphatase activities in neonatal pups. *Am J Vet Res.* **52**, 1991, 499–504.
- \*SA Center, K Warner, J Corbett, et al.: Protein invoked by vitamin K absence and clotting times in clinically ill cats. *J Vet Intern Med.* **14**, 2000, 292–297.
- RA Chamuleau, DK Bosman: Liver regeneration. *Hepatogastroenterology.* **35**, 1988, 309–312.
- LJ Counsell, JH Lumsden: Serum bile acids: Reference values in healthy dogs and comparison of two kit methods. *Vet Clin Pathol.* **17**, 1988, 71–74.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

AM Craig, EG Pearson, K Rowe: Serum bile acid concentrations in clinically normal cattle: Comparison of type, age, and stage of lactation. *Am J Vet Res.* **53**, 1992, 1784–1786.

\*AR Crawford, X Lin, JM Crawford: The normal adult human liver biopsy: A quantitative reference standard. *Hepatology.* **28**, 1998, 323–331.

AP Day, MD Feher, R Chopra, et al.: Triglyceride fatty acid chain length influences the post prandial rise in serum intestinal alkaline phosphatase activity. *Ann Clin Biochem.* **29**, 1992, 287–291.

ME De Broe, VO Van Hoof: Multiple forms of alkaline phosphatase in plasma of hemodialysis patients. *Clin Chem.* **37**, 1991, 783–784.

JJ De Bruijne, J Rothuizen: The value of serum bile acid and GLDH in the screening for canine liver function disorders. In Blackmore, DJ, Evans, GO, Sommer, H, et al. (Eds.): *Animal clinical biochemistry*. 1988, Cambridge University Press, Cambridge, 175–180.

\*DG Doherty, C O'Farrelly: Innate and adaptive lymphoid cells in the human liver. *Immunol Rev.* **174**, 2000, 5–20.

R Elialim, A Mahmood, DH Alpers: Rat intestinal alkaline phosphatase secretion into lumen and serum is coordinately regulated. *Biochim Biophys Acta.* **1091**, 1991, 1–8.

\*AA Ernst, ML Haynes, TG Nick, et al.: Usefulness of the blood urea nitrogen/creatinine ratio in gastrointestinal hemorrhage. *Am J Emerg Med.* **17**, 1999, 70–72.

\*MB Fisher, MF Paine, TJ Strelevitz, et al.: The role of hepatic and extrahepatic UDP-glucuronosyltransferases in human drug metabolism. *Drug Metab Rev.* **33**, 2001, 273–297.

A Francavilla, KA Porter, J Benichow, et al.: Liver regeneration in the dog: Morphologic and chemical changes. *J Surg Res.* **25**, 1978, 409–419.

\*JM Gagne, DJ Weiss, PJ Armstrong: Histopathologic evaluation of feline inflammatory liver diseases. *Vet Pathol.* **33**, 1996, 521–526.

FB Garry, MJ Fettman, CR Curtis, et al.: Serum bile acid concentrations in dairy cattle with hepatic lipodosis. *J Vet Intern Med.* **8**, 1994, 432–438.

\*E Gaudio, L Pannarale, P Onori, et al.: A scanning electron microscopic study of liver microcirculation disarrangements in experimental rat cirrhosis. *Hepatology.* **17**, 1993, 477–485.

SD Gilson, SJ Withrow, SL Wheeler, et al.: Pheochromocytoma in 50 dogs. *J Vet Intern Med.* **8**, 1994, 228–232.

DL Golden, JS Spano, RC Wilson, et al.: Application of an enzyme-multiplied immunoassay technique for determination of caffeine elimination kinetics as a test of liver function in clinically normal dogs. *Am J Vet Res.* **55**, 1994, 790–794.

CM Haukey, MG Hart: Fibrinogen levels in mammals suffering from bacterial infections. *Vet Rec.* **121**, 1987, 519–521.

WE Hoffmann, N Everds, M Pignatello, et al.: Automated and semiautomated analysis of rat alkaline phosphatase isoenzymes. *Toxicol Pathol.* **22**, 1994, 633–638.

BS Horney, AJ Farmer, DJ Honor, et al.: Agarose gel electrophoresis of alkaline phosphatase isoenzymes in the serum of hyperthyroid cats. *Vet Clin Pathol.* **23**, 1994, 98–102.

K Ishak: Hepatic histopathology. In Schiff, L, Schiff, E (Eds.): *Diseases of the liver*. 7th ed., 1993, JB Lippincott Co, Philadelphia, 145.

R Jalan, PC Hayes: Review article: Quantitative tests of liver function. *Aliment Pharmacol Ther.* **9**, 1995, 263–270.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- M Kuhlenschmidt, W Hoffmann, M Rippey: Glucocorticoid hepatopathy: Effect on receptor-mediated endocytosis of asialoglycoproteins. *Biochem Med Metab Biol.* **46**, 1991, 152–168.
- T Kuwana, SB Rosalki: Intestinal variant alkaline phosphatase in plasma in disease. *Clin Chem.* **36**, 1990, 1918–1921.
- WW Lamm, CV GreenWay: Conceptual review of the hepatic vascular bed. *Hepatology.* **7**, 1987, 952–963.
- DF Lawler, DG Keltner, WE Hoffman, et al.: Benign familial hyperphosphatasemia in Siberian huskies. *Am J Vet Res.* **57**, 1996, 612–617.
- \*MA Leonis, K Toney-Earley, Degen, SJF, et al.: Deletion of the Ron receptor tyrosine kinase domain in mice provides protection from endotoxin-induced acute liver failure. *Hepatology.* **36**, 2002, 1053–1060.
- \*MacSween, RNM, VJ Desmet, T Roskams, et al.: Developmental anatomy and normal structure. In MacSween, RNM, Burt, AD, Portmann, BC, et al. (Eds.): *Pathology of the liver*. 4th ed., 2002, Churchill Livingstone, Philadelphia, 1–66.
- \*RW Mahley, KH Weisgraber, T Innerarity: Canine lipoproteins and atherosclerosis. *Circ Res.* **35**, 1974, 713–733.
- \*F Marra: Hepatic stellate cells and the regulation of liver inflammation. *J Hepatol.* **31**, 1999, 1120–1130.
- \*BN Melgert, P Olinga, Van Der Laan, JMS, et al.: Targeting dexamethasone to Kupffer cells: Effects on liver inflammation and fibrosis in rats. *Hepatology.* **34**, 2001, 719–728.
- \*DJ Meyer, MB Thompson, DF Senior: Use of ursodeoxycholic acid in a dog with chronic hepatitis: Effects on serum hepatic tests and endogenous bile acid composition. *J Vet Intern Med.* **11**, 1997, 195–197.
- \*DJ Meyer: The pathophysiology of retained bile acids. *Eur J Comp Gastroenterol.* **3**, 1998, 5–11.
- \*DJ Meyer: The liver. In Raskin, RE, Meyer, DJ (Eds.): *Atlas of canine and feline cytology*. 2001, WB Saunders Co, Philadelphia, 231–252.
- T Morimoto, T Tsujinaka, M Yano, et al.: Regulation of albumin synthesis after hepatectomy and in the acute inflammation phase of rat liver. *J Nutr Biochem.* **6**, 1995, 522–527.
- \*B Mortensen, N Huseby: Clearance of circulating gamma-glutamyltransferase by the asialoglycoprotein receptor. Enzyme forms with different sialic acid content are eliminated at different clearance rates and without apparent desialylation. *Clin Chim Acta.* **258**, 1997, 47–58.
- \*ME Mount, BU Kim, PH Kass: Use of a test for proteins induced by vitamin K absence or antagonism in diagnosis of anticoagulant poisoning in dogs: 325 cases (1987-1997). *J Am Vet Med Assoc.* **222**, 2003, 194–198.
- F Muller, JW Tyler, SM Parish, et al.: Stability of  $\gamma$ -glutamyltransferase activity in calf sera after refrigerated or frozen storage. *Am J Vet Res.* **58**, 1997, 354–355.
- \*S Norris, C Collins, DG Doherty, et al.: Resident human hepatic lymphocytes are phenotypically different from circulating lymphocytes. *J Hepatol.* **28**, 1998, 84–90.
- GK Ogilvie, LM Walters, SH Greeley, et al.: Concentration of alpha 1-acid glycoprotein in dogs with malignant neoplasia. *J Am Vet Med Assoc.* **203**, 1993, 1144–1146.
- M Panteghini: Benign inherited hyperphosphatasemia of intestinal origin: Report of two cases and a brief review of the literature. *Clin Chem.* **37**, 1991, 1449–1452.
- NJ Pappas, Jr.: Source of increased serum aspartate and alanine aminotransferase: Cycloheximide effect on carbon tetrachloride hepatotoxicity. *Clin Chim Acta.* **154**, 1986, 181–190.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- \*KP Patil, NR Rangnekar: Gamma-glutamyltransferase activity in human milk. *Clin Chem.* **28**, 1982, 1724–1725.
- EG Pearson, AM Craig, K Rowe: Variability of serum bile acid concentrations over time in dairy cattle, and effect of feed deprivation on the variability. *Am J Vet Res.* **53**, 1992, 1780–1783.
- \*L Phillips, Tappe, JP, R Lyman, et al.: Hepatic microvascular dysplasia in dogs. *Prog Vet Neurol.* **7**, 1996, 88–96.
- \*Ristic, JME, IK Ramsey, FM Heath, et al.: The use of 17-hydroxyprogesterone in the diagnosis of canine hyperadrenocorticism. *J Vet Intern Med.* **16**, 2002, 433–439.
- \*D Rockey: The cellular pathogenesis of portal hypertension: Stellate cell contractility, endothelin and nitric oxide. *Hepatology.* **25**, 1997, 2–5.
- J Rothuizen, R de Vries-Chalmers Hoynck van Papendrecht, WE van den Brom: Post prandial and cholecystokinin-induced emptying of the gall bladder in dogs. *Vet Rec.* **126**, 1990, 505–507.
- J Rothuizen, WE van den Brom, J Fevery: The origins and kinetics of bilirubin in healthy dogs in comparison with man. *J Hepatol.* **15**, 1992, 25–34.
- J Rothuizen, T van den Ingh: Covalently protein-bound bilirubin conjugates in cholestatic disease in dogs. *Am J Vet Res.* **49**, 1988, 702–704.
- \*A Reuben: There is nothin' like a dame. *Hepatology.* **35**, 2002, 983–985.
- PK Saini, DW Webert: Application of acute phase reactants during antemortem and postmortem meat inspection. *J Am Vet Med Assoc.* **198**, 1991, 1898–1901.
- RK Sanecki, WE Hoffmann, HB Gelberg, et al.: Subcellular location of corticosteroid-induced alkaline phosphatase in canine hepatocytes. *Vet Pathol.* **24**, 1987, 296–301.
- \*T Schermerhorn, SA Center, NL Dykes, et al.: Characterization of microvascular dysplasia in a kindred of Cairn terriers. *J Vet Intern Med.* **10**, 1996, 219–230.
- \*P Schirmacher, A Geerts, A Pietrangelo, et al.: Hepatocyte growth factor/hepatopoietin A is expressed in fat-storing cells from rat liver but not myofibroblast-like cells derived from fat-storing cells. *Hepatology.* **15**, 1992, 5–11.
- \*V Shah, G Garcia-Cardena, WC Sessa, et al.: The hepatic circulation in health and disease: Report of a single topic symposium. *Hepatology.* **27**, 1998, 279–288.
- de Shepper, J, van der Stock, J: Influence of sex on the urinary bilirubin excretion at increased free plasma haemoglobin levels in whole dogs and in isolated normo-thermic perfused dog kidneys. *Experientia.* **27**, 1971, 1264–1265.
- S Shuichi, M Ochi, T Horiuchi, et al.: Intestinal type alkaline phosphatase hyperphosphatasemia associated with liver cirrhosis. *Clin Chim Acta.* **210**, 1992, 63–73.
- \*KW Simpson, DJ Meyer, A Boswood, et al.: Iron status and erythrocyte volume in dogs with congenital portosystemic vascular anomalies. *J Vet Intern Med.* **11**, 1997, 14–19.
- PA Siraganian, JJ Mulvihill, RA Mulivor, et al.: Benign familial hyperphosphatasemia. *JAMA.* **261**, 1989, 1310–1312.
- PF Solter, WE Hoffmann, MD Chambers, et al.: Hepatic total 3 alpha-hydroxy bile acids concentration and enzyme activities in prednisone-treated dogs. *Am J Vet Res.* **55**, 1994, 1086–1092.
- ME Stark, JH Szurszewski: Role of nitric oxide in gastrointestinal and hepatic function and disease. *Gastroenterology.* **103**, 1992, 1928–1949.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

- \*JM Steiner, DA Williams, SE Bunch: Evaluation of urine sulfated and nonsulfated bile acids as a diagnostic test for liver disease in dogs [letter]. *J Am Vet Med Assoc.* **223**, 2003, 429–430.
- \*JM Steiner, DA Williams, DC Twedt: Urine sulfated and nonsulfated bile acids as a diagnostic test for liver disease in cats [letter]. *J Vet Intern Med.* **17**, 2003, 605–606.
- \*T Stokol, H Erb: The apo-enzyme content of aminotransaminases in healthy and diseased domestic animals. *Vet Clin Pathol.* **27**, 1998, 71–78.
- RJ Sutherland, HS Deol, PJ Hood: Changes in plasma bile acids, plasma amino acids, and hepatic enzyme pools as indices of functional impairment in liver-damaged sheep. *Vet Clin Pathol.* **21**, 1992, 51–56.
- \*J Taboada, DJ Meyer: Cholestasis associated with extrahepatic bacterial infection in five dogs. *J Vet Intern Med.* **3**, 1989, 216–221.
- \*HF Teutsch, D Schuerfeld, E Groezinger: Three-dimensional reconstruction of parenchymal units in the liver of the rat. *Hepatology.* **29**, 1999, 494–505.
- \*D Trainor, SA Center, JF Randolph, et al.: Urine sulfated and nonsulfated bile acids as a diagnostic test for liver disease in cats. *J. Vet Intern Med.* **17**, 2003, 145–153.
- \*M Trauner, PJ Meier, JL Boyer: Molecular pathogenesis of cholestasis. *N Engl J Med.* **339**, 1998, 1217–1227.
- \*D Vermijlen, D Luo, B Robaye, et al.: Pit cells (hepatic natural killer cells) of the rat induce apoptosis in colon carcinoma cells by the perforin/granzyme pathway. *Hepatology.* **29**, 1999, 51–56.
- \*Villeneuve, J-P, M Dagenais, Huet, P-M, et al.: The hepatic microcirculation in the isolated perfused human liver. *Hepatology.* **23**, 1996, 24–31.
- T Washizu, I Tomoda, JJ Kaneko: Serum bile acid composition of the dog, cow, horse, and human. *J Vet Med Sci.* **53**, 1991, 81–86.
- \*DJ Weiss, JM Gagne, PJ Armstrong: Characterization of portal lymphocytic infiltrates in feline liver. *Vet Clin Pathol.* **24**, 1995, 91–95.
- \*DJ Weiss, JM Gagne, PJ Armstrong: Relationship between inflammatory hepatic disease and inflammatory bowel disease, pancreatitis, and nephritis in cats. *J Am Vet Med Assoc.* **209**, 1996, 1114–1116.
- \*CE Wiedmeyer, PF Solter, WE Hoffmann: Alkaline phosphatase expression in tissues from glucocorticoid-treated dogs. *Am J Vet Res.* **63**, 2002, 1083–1088.
- \*CE Wiedmeyer, PF Solter, WE Hoffmann: Kinetics of mRNA expression of alkaline phosphatase isoenzymes in hepatic tissues from glucocorticoid-treated dogs. *Am J Vet Res.* **63**, 2002, 1089–1095.
- \*M Winnock, M Garcia Barcina, B Lukomska, et al.: Human liver-associated lymphocytes: An overview. *J Gastroenterol Hepatol.* **10**, 1995, S43–S46.
- S Yamamoto, T Shida, T Okimura, et al.: Determination of C-reactive protein in serum and plasma from healthy dogs and dogs with pneumonia by ELISA and slide reversed passive latex agglutination test. *Vet Q.* **16**, 1994, 74–77.
- M Zoli, H Marchesini, A Melli: Evaluation of liver volume and liver function following hepatic resection in man. *Liver.* **6**, 1986, 286–291.

## 11.9 SKELETAL MUSCLE

- M Aktas, D Auguste, HP Lefebvre, et al.: Creatine kinase in the dog: A review. *Vet Res Commun.* **17**, 1993, 353–369.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

AJ Fascetti, GE Mauldin, GN Mauldin: Correlation between serum creatinine kinase activities and anorexia in cats. *J Vet Intern Med.* **11**, 1997, 9–13.

DR Finco, SA Brown, SL Vaden, et al.: Relationship between plasma creatinine concentration and glomerular filtration in dogs. *J Vet Pharmacol Ther.* **18**, 1995, 418–421.

BA Valentine, JT Blue, SM Shelley, et al.: Increased serum alanine aminotransferase activity associated with muscle necrosis in the dog. *J Vet Intern Med.* **4**, 1990, 140–143.

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## 12 Chapter 11 Evaluation of Exocrine Pancreatic and Intestinal Disorders

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*Chance favors the prepared mind.*

**Louis Pasteur, 1822-1865, French chemist and bacteriologist who founded modern microbiology, invented the process of pasteurization, and developed vaccines for anthrax, rabies, and chicken cholera.**

### 12.1 EXOCRINE PANCREAS

Pancreatic fluid is composed of digestive enzymes secreted by acini and ductal secretions of water and sodium bicarbonate. The latter neutralizes hydrochloric acid emptied from the stomach for optimal digestive activities. The exocrine pancreatic tissue synthesizes approximately 20 digestive enzymes, colipase, and a trypsin inhibitor. The enzymes are stored in zymogen granules. Digestive enzymes are trapped in an inactive form, with the exception of amylase and lipase. Cholecystokinin, released from the mucosal cells of the duodenum and upper jejunum on stimulation of luminal protein and fat, prompts the discharge of the granule contents. Trypsin, chymotrypsin, carboxypolypeptidase, and several elastases and nucleases are the principal proteolytic enzymes. The first three enzymes are synthesized as inactive forms and are activated only after reaching the intestine. The concurrent secretion of the trypsin inhibitor prevents the autocatalytic activation of these potent proteolytic enzymes within the pancreas. Amylase digests complex carbohydrates, forming disaccharides. Lipase hydrolyzes neutral fat, forming fatty acids and monoglycerides. Levels of amylase and lipase in pancreatic tissue are generally high.

#### 12.1.1 Pancreatitis (Fig. 11-1; Case 11-1, see also Table 8-1)

Autodigestion of the pancreas is referred to as *acute pancreatitis*. A variety of errors in intraacinar cell processes result in colocalization of the stored inactive enzymes and lysosomal enzymes, which overwhelms the protective mechanisms. Increased pancreatic duct permeability appears to be a contributory factor. Premature activation of enzymes is principally responsible for the intraorgan escape of activated enzymes. Specific causes for initiation of the pathophysiologic events, with the exception of trauma, are poorly defined. Hyperlipemia, hypercalcemia, and exposure to organophosphates appear to be risk factors.

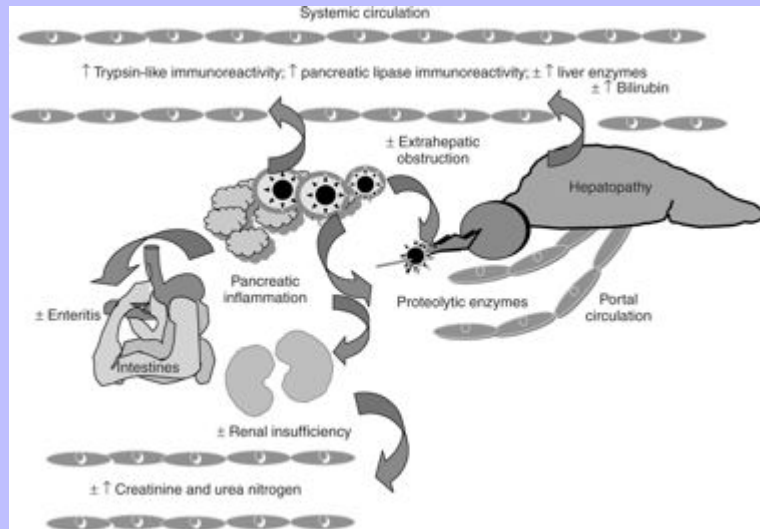
Pancreatitis may be focal or diffuse; may involve peripancreatic tissues, causing peripancreatic fat necrosis and focal peritonitis; may cause disease at distinct sites such as the liver (hepatopathy), intestine (enteritis or colitis), kidneys (renal failure), and lungs (pleural effusions); or may initiate multiple organ failure syndrome. The release of proteases into the circulation is initially inactivated by  $\alpha_2$ -macroglobulin and  $\alpha_1$ -proteinase inhibitor, both of which can be overwhelmed by the excess enzymes. Trypsin initiates a cascade of events that result in the formation of vasoactive kinins that promote neutrophil accumulation.

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Fig. 11-1



Acute pancreatitis. Premature activation of enzyme precursors within the exocrine pancreas causes autodigestion of the organ. Damaged cells may initially release enzymes into the peritoneal cavity, with subsequent absorption by diaphragmatic lymphatics, rather than directly into the circulation. Variable pancreatic or peripancreatic disease may impair this route of absorption and may be one explanation for the relatively poor diagnostic sensitivity of traditional lipase and amylase levels compared with their consistent increase after the induction of experimental pancreatitis. A greater than threefold increase in one or both of these traditional biochemical markers for acute pancreatitis in dogs supports the diagnosis. Neither has diagnostic value for detection of acute pancreatitis in cats. The measurement of pancreatic lipase immunoreactivity in the serum by a radioimmunoassay appears to be sensitive and specific for the diagnosis of acute pancreatitis in dogs and cats. Trypsin-like immunoreactivity (TLI) is specific for the pancreas, and an increase in the circulating TLI in the dog or cat is indicative of acute pancreatitis. A final diagnosis of acute pancreatitis is based on the integration of clinical, laboratory, and radiographic or ultrasonographic findings.

### 12.1.2 Amylase (3.2.1.1; Amy) and Lipase (EC 3.1.1.3; Lip)

The diagnosis of acute pancreatitis is based on clinical, laboratory, and radiographic or ultrasonographic findings. Clinical signs of repeated vomiting and abdominal pain in dogs are historically helpful in making the diagnosis but are often not noted in cats. Measurement of the circulating amylase and lipase activities after induction of experimental pancreatitis in dogs shows fairly consistent increases that last several days but has notoriously poor sensitivity and specificity when determined by traditional methods in animals with naturally occurring disease. An increase of greater than threefold in one or both enzyme levels supports a clinical diagnosis of acute pancreatitis. Renal failure can increase one or both enzyme levels two- to threefold.

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Hyperamylasemia caused by the formation of macroamylase (amylase bound to circulating immune complexes) has been reported in dogs with leishmaniasis. However, the traditional amylase and lipase tests lack diagnostic value in cats. The measurement of trypsin-like immunoreactivity (TLI) in dogs and cats appears to improve the biochemical detection of acute pancreatitis, but the “window” of diagnostic value may be limited, perhaps only

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24 to 36 hours, after the initial attack of pancreatic inflammation. Renal failure increases circulating TLI. The measurement of circulating pancreatic lipase by a specific radioimmunoassay appears to be highly sensitive for the diagnosis of acute pancreatitis in dogs. Consultation with the clinical pathology laboratory for the most current assessment is recommended for the application of these tests in dogs and cats.

## 12.1.2.1 BOX 11-1 Causes of Hyperlipidemia and/or Hyperamylasemia

Pancreatic inflammation, necrosis, or neoplasia

Chronic renal insufficiency

Macroamylasemia

Glucocorticoids (slight, lipase only)

Intestinal perforation

Ultrasonographic pancreatic changes are an important contribution to the diagnosis of acute pancreatitis in dogs and cats. Aspiration biopsy and cytologic examination of a lesion caused by acute pancreatitis and identified with ultrasonography can yield a nonseptic exudate. The microscopic findings will consist of a granular basophilic proteinaceous background that contains inflammatory cells. The most prominent cells are neutrophils, both nondegenerate and mushy-appearing forms because of the effects of proteolytic enzymes, and lesser numbers of foamy macrophages. The lipase activity of the exudate can yield values that are many-fold higher than the circulating lipase activity measured by standard methods.

Abnormal hepatic enzyme levels and liver function, reflected as hyperbilirubinemia, are common findings in association with acute pancreatitis. Histopathologic changes of vacuolar hepatopathy, hepatocellular cytolysis, lymphocytic or mixed cell cholangitis, and/or hepatocellular cholestasis are probably caused by the release of potent proteolytic enzymes into the portal circulation. An experimental study in dogs indicated that the severity of symptoms was directly related to the magnitude of the underlying pancreatic disease. Mechanical obstruction of the common bile duct may occur as a result of swelling of the inflamed peripancreatic or pancreatic tissue. The observation of abnormal liver test results in a dog with acute, repetitive vomiting should prompt the differential diagnosis of acute pancreatitis. The abnormal liver test results will return toward normal within 7 to 10 days after resolution of the pancreatitis.

In a case of acute pancreatitis, persistence of hyperbilirubinemia beyond 10 days, especially if the patient's condition appears to have improved clinically, implies an extrahepatic obstructive process for which a diagnosis should be pursued with ultrasonography. Cats with acute pancreatitis often have liver test results suggestive of cholestatic liver disease but often do not vomit or demonstrate abdominal pain. Concurrent conditions in cats include hepatic lipidosis, cholangitis, enteritis, and diabetes mellitus.

## 12.1.3 Trypsin (3.4.21.4)

Trypsin is a serine proteinase that hydrolyzes the peptide bonds of lysine or arginine. The pancreatic acinar cells synthesize it in the form of the inactive proenzyme trypsinogen. On secretion of the pancreatic fluid into the intestinal tract, enterokinase converts trypsinogen to the active form, trypsin, where it functions as a digestive enzyme. With pancreatic injury, autocatalysis can result in the formation and release of trypsin into the circulation, and trypsin can be measured as a specific marker of pancreatic disease. Increased circulating

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levels of trypsin have been measured in horses with intestinal obstruction (acute abdomen), and concurrent light and electron microscopic alterations were observed in pancreatic tissue.

## 12.2 TESTS FOR ASSESSING MALDIGESTION AND MALABSORPTION

The exocrine pancreas and intestinal tract are inextricably intertwined functionally for the digestion and absorption of ingested nutrients. Diseases involving either organ often cause nonlocalizing signs of vomiting, diarrhea, and/or weight loss of variable intensity. The evaluation of pancreatic and intestinal function is challenging, and many early tests are no longer considered diagnostically reliable. These include Sudan staining for fecal fat (before and after addition of acetic acid), iodine staining for fecal starch, assessment for fecal muscle fibers, fecal trypsin–gel tube or radiographic film digestion, the plasma turbidity test, and xylose absorption. The oral glucose tolerance test has value in assessment of endocrine function of the pancreas.

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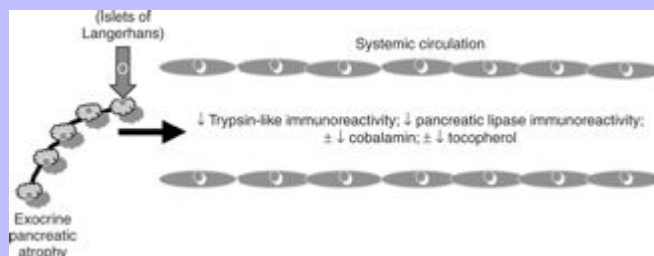
The use of a screening profile provides a biochemical foundation for differentiating exocrine pancreatic insufficiency (maldigestion) from primary intestinal tract disease. Exocrine pancreatic insufficiency can cause alterations in the proximal intestine that result in maldigestion, further confounding evaluation. One screening profile consists of measuring circulating TLI and folate and cobalamin levels. A reduction in TLI is generally diagnostic of exocrine pancreatic insufficiency. A rise in the folate level and/or reduction in the cobalamin level coincident with a TLI within reference range implicates disease involving the proximal small intestine.  $\alpha_1$ -Proteinase inhibitor can be measured in the feces as a biochemical marker of protein-losing intestinal disease if the circulating albumin and globulin values are reduced. Microscopic examination of an intestinal biopsy specimen and/or fecal sample is necessary to make a definitive diagnosis, once primary intestinal disease is suspected. The more common diseases are discussed.

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### 12.2.1 Maldigestion: Exocrine Pancreatic Insufficiency (Fig. 11-2)

Circulating trypsinogen is a pancreas-specific marker. It is measured with a radioimmunoassay and expressed as TLI. It is a sensitive and specific biochemical marker for assessment of exocrine pancreatic function. A value that is less than the reference range is diagnostic of exocrine pancreatic insufficiency in dogs and cats. The majority of the circulating lipase and amylase in the dog is not of pancreatic origin. Neither enzyme level, when measured by traditional methods, is lower than the reference range after complete pancreatectomy in dogs. Consequently, measurement of lipase and amylase levels by standard methods is not a useful screening test for the evaluation of exocrine pancreatic insufficiency. A reduction of pancreatic lipase immunoreactivity measured in the serum by a radioimmunoassay is indicative of exocrine pancreatic insufficiency. A radial enzyme diffusion method for accurate measurement of fecal proteolytic activity with an azocasein substrate is a cumbersome but useful test for the diagnosis of exocrine pancreatic insufficiency. Collection of three fecal specimens, on different days, is recommended. The specimens can be frozen at  $-20^{\circ}\text{C}$ .

Fig. 11-2



Exocrine pancreatic insufficiency. The absence or loss of pancreatic acinar cells is most commonly due to atrophy and much less frequently a consequence of chronic pancreatitis. Exocrine pancreatic acinar atrophy can manifest at any age and in many breeds, but it is prevalent in German shepherds and collies. A reduction in trypsin-like immunoreactivity (TLI) is a sensitive and specific indicator of this loss of digestive function, as is a reduction in the pancreatic lipase *immunoreactivity* measured by radioimmunoassay. Concomitant bacterial overgrowth in the proximal small intestine is common, resulting in increased folate levels in the circulation. Concurrent reductions in circulating cobalamin and tocopherol levels may be marked, and consideration should be given to their contribution to effective therapeutic management. Reductions in circulating levels of other fat-soluble vitamins A, D, and K often do not manifest clinically, although treatment for a reduction in vitamin K level should be considered if a concurrent coagulopathy is identified. The endocrine tissue (islets of Langerhans) is usually not affected and glucose homeostasis is not impaired.

No pancreatic enzyme supplements should be administered for 1 week before the use of these tests to obviate potential negative feedback inhibition of a healthy pancreas. Consultation with the clinical pathology laboratory for the most current recommendations for the use of these tests and the interpretation of the results is recommended.

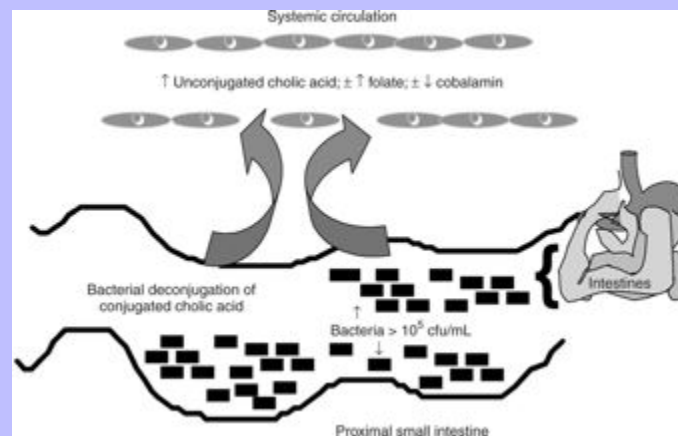
## 12.3 **INTESTINAL DISORDERS: SMALL INTESTINAL BACTERIAL OVERGROWTH (Fig. 11-3) AND PROTEIN-LOSING ENTEROPATHY (Fig. 11-4, Box 11-2; see also Fig. 10-6)**

Selected types of intestinal bacteria can synthesize and secrete some vitamins with subsequent absorption into the circulation. Folate is one of these bacterial products. Bacterial overgrowth causes an increased intraluminal folate level that ultimately increases the level in the circulation. In contrast, coincident with bacterial overgrowth, utilization of cobalamin is increased and/or its absorption is impaired with an eventual reduction of its level in the circulation. Deconjugation of a small proportion of conjugated bile acids by enteric bacterial flora is a physiologically normal process. With bacterial overgrowth, the deconjugation process is amplified and increases the intraluminal levels of unconjugated bile acids. Unconjugated bile acids are passively absorbed along most of the intestinal tract, in contrast to receptor-mediated uptake of conjugated bile acids in the ileum. Unconjugated bile acids are not as efficiently removed from the circulation as conjugated bile acids. Both factors facilitate the increase in the circulating levels of unconjugated bile acids. An increase in the circulating level of unconjugated cholic acid is associated with bacterial overgrowth in the proximal small intestine. Although the magnitude of the increase does not usually cause the total bile acid concentration (mostly conjugated forms) to increase above the upper limit of the reference range, in some dogs with bacterial overgrowth, total bile acid values that exceed the upper limit of the reference range by as much as three- to fivefold have been documented without notable

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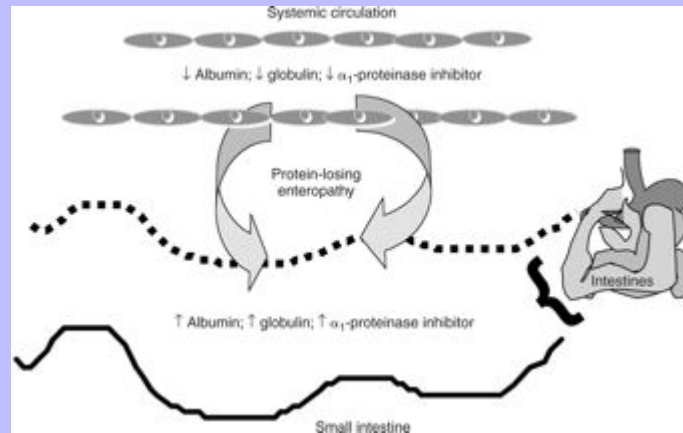
concurrent liver disease or abnormal portal circulation. An increased incidence of bacterial overgrowth has been observed in apparently healthy beagles and German shepherds. The documentation of small intestinal bacterial overgrowth in cats is being investigated. The defining criteria will probably differ from those in dogs. Cobalamin deficiency and cobalamin-responsive diarrhea are recognized in cats.

Fig. 11-3



Small intestinal bacterial overgrowth. An overgrowth of bacteria in the proximal small intestine is generally defined as a quantitated bacterial count of greater than  $10^5$  colony-forming units (CFU) per milliliter of canine duodenal fluid. Enteric bacteria synthesize and secrete folate. Bacterial overgrowth causes an increased intraluminal folate level that ultimately increases the level in the circulation. In contrast, there is increased utilization of cobalamin by the altered intestinal bacterial flora and/or its absorption is impaired and ultimately results in a reduction of the circulating cobalamin level. As biochemical markers of bacterial overgrowth of the proximal intestine, neither the folate level nor the cobalamin level has a high sensitivity compared with quantitated culture. Deconjugation of a small proportion of conjugated bile acids by enteric bacterial flora is a physiologically normal component of bile acid enterohepatic circulation. With bacterial overgrowth, the deconjugation process is amplified and raises the intraluminal levels of unconjugated bile acids. In contrast to receptor-mediated uptake of conjugated bile acids in the ileum, unconjugated bile acids are generally passively absorbed along the intestinal tract. They are not as efficiently removed from the circulation as conjugated bile acids. Both factors facilitate the increase in the circulating levels of unconjugated bile acids. The measurement of unconjugated cholic acid (UCA) increases the biochemical sensitivity for the detection of proximal intestinal bacterial overgrowth. The contribution of the increased unconjugated cholic acid concentration in the circulation is generally not high enough to cause the total bile acid concentration to increase above the reference range. However, some dogs with bacterial overgrowth will have total bile acid values that exceed the upper reference range by as much as three- to fivefold without concurrent notable liver disease or abnormal portal circulation. There is an apparent increased incidence of bacterial overgrowth in clinically healthy beagles, notably those used in studies by the pharmaceutical industry, and German shepherd dogs. The documentation of small intestinal bacterial overgrowth in cats is currently being explored and documented. The defining criteria will probably differ from those in the dog. Cobalamin deficiency and cobalamin-responsive diarrhea are recognized in cats.

Fig. 11-4



Protein-losing enteropathy. Disease that alters the integrity of the intestinal mucosal barrier can result in the loss of plasma constituents, notably proteins. There is often a concomitant loss of albumin and globulins. Measurement of fecal radiolabeled albumin after intravenous injection is the definitive test for documenting protein loss. A clinically useful test for detecting protein-losing intestinal disease is the measurement of  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ -antitrypsin) in the feces.  $\alpha_1$ -Proteinase inhibitor ( $\alpha_1PI$ ) has a molecular weight similar to that of albumin and is resistant to luminal digestion. Microscopic examination of an intestinal biopsy specimen and/or a fecal sample is necessary to define the disease.

## 12.3.1 BOX 11-2 Common Causes of Protein-Losing Enteropathy (Generally Hypoalbuminemia and Hypoglobulinemia)

Lymphangiectasia

Inflammation (lymphoplasmacytic, eosinophilic, granulomatous)

Basenji enteropathy (hypoalbuminemia with hyperglobulinemia)

Infection (parasites, fungi<sup>\*</sup>)

Neoplasia

<sup>\*</sup> Animals with histoplasmosis may have normoglobulinemia or hyperglobulinemia.

## 12.4 ADDITIONAL READING

KG Akol, RJ Washabau, HM Saunders, et al.: Acute pancreatitis in cats with hepatic lipidosis. *J Vet Intern Med.* 7, 1993, 205–209.

198

RH Baker: Acute necrotizing pancreatitis in a horse. *J Am Vet Med Assoc.* 172, 1978, 268–270.

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# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- JR Bellah, G Bell: Serum amylase and lipase activities after exploratory laparotomy in dogs. *Am J Vet Res.* **50**, 1989, 1638–1640.
- AK Cook, EB Breitschwerdt, JF Levine, et al.: Risk factors associated with acute pancreatitis in dogs: 101 cases (1985–1990). *J Am Vet Med Assoc.* **203**, 1993, 673–679.
- M Corazza, R Tognetti, G Guidi, et al.: Urinary alpha-amylase and serum macroamylase activities in dogs with proteinuria. *J Am Vet Med Assoc.* **205**, 1994, 438–440.
- C Fittschen, Bellamy, JEC: Prednisone treatment alters the serum amylase and lipase activities in normal dogs without causing pancreatitis. *Can J Comparative Med.* **48**, 1984, 136–140.
- AJ German, MJ Day, CG Ruaux, et al.: Comparison of direct and indirect tests for small intestinal bacterial overgrowth and antibiotic-responsive diarrhea in dogs. *J Vet Intern Med.* **17**, 2003, 33–43.
- S Grulke, G Deby-Dupont, D Cassart, et al.: Pancreatic injury in equine acute abdomen evaluated by plasma trypsin activity and histopathology of pancreatic tissue. *Vet Pathol.* **40**, 2003, 8–13.
- K Hibbetts, B Hines, D Williams: An overview of proteinase inhibitors. *J Vet Intern Med.* **13**, 1999, 302–308.
- RC Hill, TJ Van Winkle: Acute necrotizing pancreatitis and acute suppurative pancreatitis in the cat. *J Vet Intern Med.* **7**, 1993, 25–33.
- RM Jacobs: The origins of canine serum amylases and lipases. *Vet Pathol.* **26**, 1989, 525–527.
- AL Jensen: Serum fructosamine as a screening test for diabetes mellitus in non-healthy middle-aged to older dogs. *Zentralbl Veterinarmed A.* **41**, 1994, 480–484.
- ND Karanjia, AL Widdison, A Jehanli, et al.: Assay of trypsinogen activation in the cat experimental model of acute pancreatitis. *Pancreas.* **8**, 1993, 189–195.
- BE Kitchell, DR Strombeck, J Cullen, et al.: Clinical and pathologic changes in experimentally induced acute pancreatitis in cats. *Am J Vet Res.* **47**, 1986, 1170–1173.
- TA Lutz, JS Rand, P Watt, et al.: Pancreatic biopsy in the cat. *Aust Vet J.* **71**, 1994, 223–227.
- SL Marks: Editorial: Small intestinal bacterial overgrowth in dogs—less common than you think?. *J Vet Intern Med.* **17**, 2003, 5–7.
- TM Montgomery, RW Nelson, EC Feldman, et al.: Basal and glucagon-stimulated plasma C-peptide concentrations in healthy dogs, dogs with diabetes mellitus, and dogs with hyperadrenocorticism. *J Vet Intern Med.* **10**, 1996, 116–122.
- BW Parry, MV Crisman: Serum and peritoneal fluid amylase and lipase reference values in horses. *Equine Vet J.* **23**, 1991, 390–391.
- ME Peterson, RS Taylor, DS Breco, et al.: Acromegaly in 14 cats. *J Vet Intern Med.* **4**, 1990, 192–201.
- DJ Polzin, CA Osborne, JB Stevens, et al.: Serum amylase and lipase activities in dogs with chronic primary renal failure. *Am J Vet Res.* **44**, 1983, 404–407.
- CE Reusch, MR Liehs, M Hoyer, et al.: Fructosamine. *J Vet Intern Med.* **7**, 1993, 177–182.
- B Siliart, F Stambouli: Laboratory diagnosis of insulinoma in the dog: A retrospective study and a new diagnostic procedure. *J Small Anim Pract.* **37**, 1996, 367–370.
- KW Simpson, JT Shiroma, DS Biller, et al.: Antemortem diagnosis of pancreatitis in four cats. *J Small Anim Pract.* **35**, 1994, 93–99.
- KW Simpson, JW Simpson, DB Morton, et al.: Effect of pancreatectomy on plasma activities of amylase, isoamylase, lipase, and trypsin-like immunoreactivity in dogs. *Res Vet Sci.* **51**, 1991, 78–82.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- AH Sparkes, DT Adams, PJ Cripps, et al.: Inter- and intraindividual variability of the response to intravenous glucose tolerance testing in cats. *Am J Vet Res.* **57**, 1996, 1294–1298.
- JM Steiner, TL Medinger, DA Williams: Development and validation of a radioimmunoassay for feline trypsin-like immunoreactivity. *Am J Vet Res.* **57**, 1996, 1417–1420.
- EM Streeter, E Zsombor-Murray, KE Moore, et al.: Intestinal permeability and absorption in dogs with traumatic injury. *J Vet Intern Med.* **17**, 2003, 669–673.
- Y Sugimoto, T Hayakawa, T Kondo, et al.: Peritoneal absorption of pancreatic enzymes in bile-induced acute pancreatitis in dogs. *J Gastroenterol Hepatol.* **5**, 1990, 493–498.
- SI Thoresen, WP Bredal: Clinical usefulness of fructosamine measurements in diagnosing and monitoring feline diabetes mellitus. *J Small Anim Pract.* **37**, 1996, 64–68.
- L Traverso, W Longmire, R Tompkins: A study of experimental canine pancreatitis. *J Surg Res.* **21**, 1976, 247–253.
- DA Williams: New tests of pancreatic and small intestinal function. *Comp Contin Educ Pract Vet.* **9**, 1987, 1167–1174.
- DA Williams, RM Batt: Sensitivity and specificity of radioimmunoassay of serum trypsin-like immunoreactivity for the diagnosis of canine exocrine pancreatic insufficiency. *J Am Vet Med Assoc.* **192**, 1988, 195–201.
- DA Williams, SD Reed, L Perry: Fecal proteolytic activity in clinically normal cats and in a cat with exocrine pancreatic insufficiency. *J Am Vet Med Assoc.* **197**, 1990, 210–212.

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## <sup>13</sup>Chapter 12 Evaluation of Endocrine Function, Bone and Mineral Metabolism, and Lipid Metabolism

DR. WATSON: “*What say Holmes?*”

SHERLOCK HOLMES: “... *we are suffering from a plethora of surmise, conjecture, and hypothesis. The difficulty is to detach the framework of fact—absolute undeniable fact—from the embellishments of theories and reporters. Then having established ourselves upon this sound basis, it is our duty to see what inferences may be drawn, and which are the special points upon which the whole mystery turns.*”

Arthur Conan Doyle,

*The Adventure of Silver Blaze*

### <sup>13.1</sup>ADRENAL GLANDS

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#### <sup>13.1.1</sup>Physioanatomy

Each adrenal gland consists of two structurally and functionally independent endocrine organs: the cortex and the medulla. The chromaffin tissue of the medulla is derived from neuroectoderm and is notable for its secretion of catecholamines in response to stimulation of the sympathetic nervous system. Hypoglycemia and “stress” (e.g., an acute reduction in the blood pressure) are the two most prominent stimuli. The cortex is of mesodermal origin and is comprised of three functionally distinct but not morphologically distinct layers (zones) that surround the medulla. The outer layer (zona glomerulosa) produces the mineralocorticoid, aldosterone, the principal hormone that orchestrates sodium and potassium concentrations in the maintenance of normal fluid balance and circulatory volume. The middle layer (zona fasciculata) secretes glucocorticoids, notably cortisol (corticosterone in rodents), and the inner layer of the cortex (zona reticularis) secretes sex steroids. Cortisol and aldosterone are synthesized from cholesterol and only a hydroxyl group on C-17 of the cortisol structure differentiates the two resulting in an overlap in their biologic activity. However, the potency for glucocorticoid or mineralocorticoid activity is greatest for cortisol and aldosterone, respectively. Cortisol is transported in the blood by corticosteroid-binding globulin (transcortin) and to a lesser extent by albumin. Approximately 10% is unbound (free). Aldosterone is transported predominantly by albumin with approximately 40% unbound.

#### <sup>13.1.2</sup>Cortisol Metabolism and Its Regulation (Fig. 12-1)

Glucocorticoids play a major role in glucose metabolism. Hepatic gluconeogenesis (conversion of amino acids to glycogen) is stimulated and the effects of glucagon and epinephrine are enhanced. To support this activity, glucocorticoids inhibit protein synthesis and, in excess, cause protein catabolism with subsequent breakdown and release of amino acids. The loss of mass of the abdominal muscles contributes to the “pot-bellied” appearance of dogs with cortisol excess secondary to hyperadrenocorticism. Glucocorticoids have an “anti-insulin” effect (i.e., interfere with glucose uptake and metabolism by skeletal muscle and adipose tissue). Cortisol excess should be considered in a patient with diabetes mellitus when insulin regulation is problematic.

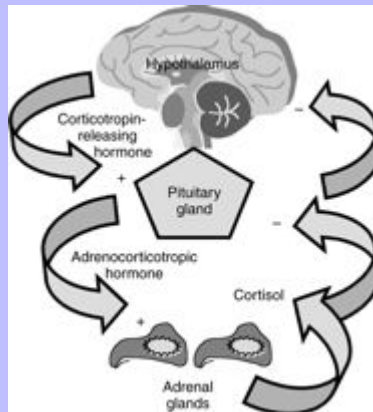
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Glucocorticoid excess, endogenous or exogenous, commonly causes excessive glycogen storage in the canine liver and abnormal hepatic enzyme test results.

Fig. 12-1



The hormonal interactions regulating the hypothalamic-pituitary-adrenal axis. A reduction of the circulating cortisol initiates the release of corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH) into the peripheral circulation and activates the adrenal glands to release cortisol. An increase in the circulating cortisol inhibits their release (negative feedback).

The hypothalamic-pituitary-adrenal axis regulates cortisol secretion by a negative feedback loop. Corticotropin-releasing hormone (CRH) from the hypothalamus stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary that then travels to the adrenal cortex to cause the secretion of cortisol. An increase in the circulating cortisol impairs the release of CRH and ACTH. The administration of exogenous glucocorticoids for medical use inhibits the release of ACTH. If the medication is abruptly discontinued before the hypothalamic-pituitary-adrenal axis establishes its function, clinical signs of lethargy and weakness may be manifested. Glucocorticoids facilitate water diuresis by enhancing the glomerular filtration rate and interfering with the activity of vasopressin (antidiuretic hormone) on the distal tubule. This is manifested clinically as polyuria. A well-known stimulus of hypothalamic-pituitary-adrenal axis resulting in an increase of the circulating cortisol is stress. Stress is a physiological reaction, such as illness or excessive physical activity, that is stimulated by an adverse event that upsets normal physiological homeostasis. In the dog, for example, a hematological reflection of the hypercortisolemia is a change in the differential white blood cell count referred to as the *corticosteroid* or *stress leukogram*. The liver of the dog is uniquely sensitive to glucocorticoids. If the stress-induced increase in circulating cortisol is of sufficient duration, an increase in the circulating alkaline phosphatase activity may occur. Concurrent stress-induced sympathoadrenal-stimulated release of excess epinephrine can contribute to changes to the hemogram or produce metabolic effects such as hyperglycemia in cats. Nonendocrine disease can confound the interpretation of the function test results designed to evaluate the hypothalamic-pituitary-adrenal axis.

### 13.1.3 Aldosterone Metabolism and Its Regulation

Aldosterone secretion is regulated by the renin-angiotensin system that involves interaction with the kidney. The juxtaglomerular cells located in the wall of the afferent glomerular arteriole synthesize and secrete rennin

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in response to several signals, such as changes in renal perfusion pressure (baroreceptor response). Renin is a proteolytic enzyme that initiates an enzymatic chain reaction by converting angiotensinogen, produced by the liver, to angiotensin I followed by action of angiotensin-converting enzyme to form angiotensinogen II. In addition to causing potent vasoconstriction, it stimulates the release of aldosterone. The potassium concentration is an important regulator of aldosterone secretion. An increase in the potassium concentration causes a secretion of aldosterone while a reduction is inhibitory.

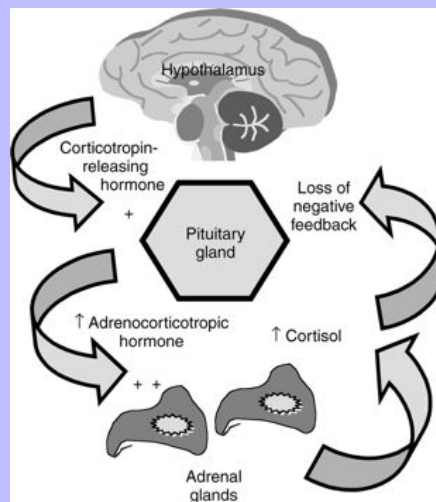
### 13.1.4 Canine Hyperadrenocorticism (Figs. 12-2 and 12-3)

Hypercortisolemia in the dog is usually caused by a pituitary tumor secreting excess ACTH. Bilateral adrenal hyperplasia occurs as a consequence of the chronic stimulation by ACTH. The remainder is due to an autonomously functioning adrenal tumor. The classic signs of hypercortisolemia are polyuria/polydipsia, polyphagia, endocrine alopecia, pot-bellied appearance, and panting. The leukogram may exhibit neutrophilia, lymphopenia, and eosinopenia. Elevated serum hepatic enzyme activities and cholesterol concentration may be measured and lipemia may be observed. A slight increase in the sodium and reduction in the potassium concentrations may be detected. Concurrent vomiting or diarrhea may amplify these changes. The urine specific gravity is often approximately 1.010 with concomitant circulating urea nitrogen and creatinine levels often within or slightly less than the reference range. Normal renal-concentrating ability is restored after establishment of the medullary-concentrating gradient. Prolonged hypercortisolemia predisposes to infections and bacterial cystitis may be detected by urinalysis. Chronic hypercortisolemia frequently suppresses thyroid-stimulating hormone (TSH; thyrotropin) secretion and changes the metabolism of the thyroid hormones, resulting in reductions in the plasma thyroxine ( $T_4$ ), free thyroxine ( $fT_4$ ), and triiodothyronine ( $T_3$ ) concentrations.

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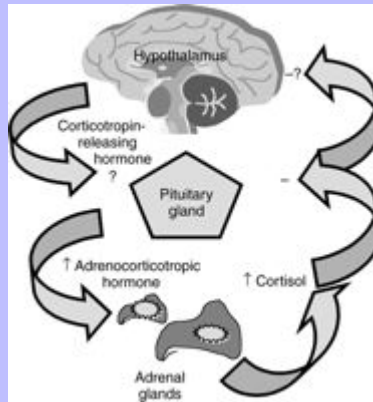
Fig. 12-2



Dysfunctional hypothalamic-pituitary-adrenal axis caused by pituitary-dependent adrenal gland hyperplasia. An excess secretion of adrenocorticotrophic hormone (ACTH) by neoplastic corticotroph cells of the pituitary gland causes marked hypertrophy and hyperplasia of the adrenal glands (zona fasciculata and reticularis), which results in the excess production and release of cortisol. Negative feedback suppression of ACTH release is lost.

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Fig. 12-3



Dysfunctional hypothalamic-pituitary-adrenal axis caused by an adrenal gland adenoma/adenocarcinoma. The functional neoplasm of the adrenal gland secretes excess cortisol into the circulation, which signals the pituitary gland to reduce the release of adrenocorticotropin-releasing hormone.

A variety of testing protocols have been developed to assess adrenal hyperfunction based on the measurement of plasma cortisol concentrations before and after administration of ACTH to stimulate the adrenal glands or dexamethasone to suppress the hypothalamic-pituitary-adrenal axis. Although tests of endocrine function are well defined, they can be affected by nonendocrine disease that complicates the interpretation of the findings. The following testing strategies provide guidance for the differential diagnosis of hyperadrenocorticism. Testing strategies and evaluation of the cortisol values should be conducted in concordance with the recommendations of the laboratory performing the assay.

The diagnostic ability of a screening test to detect a disease is related to prevalence of that disease in the general population and patient selection for testing. The impact of disease prevalence on the predictive value for the three screening tests for hyperadrenocorticism—low-dose dexamethasone suppression test, ACTH stimulation test, and urinary cortisol/creatinine ratio—is illustrated by assigning a test sensitivity and specificity of 90% to each of the three screening tests, a value that is much better than determined in clinical trials. The prevalence of hyperadrenocorticism in the general population is approximately 0.1%. Testing dogs at random would result in an extremely low predictive value for the screening test (0.01). Selection of the patient to be tested based on the number of criteria determined in the history (clinical signs), findings on the physical examination, and preliminary laboratory findings (increased alkaline phosphatase [ALP]) that are suggestive of hyperadrenocorticism dramatically improves the diagnostic ability of the screening test. Increasing the rigor of the selection criteria to “concentrate” the prevalence of the disease in a smaller testing population to 25% and, with greater scrutiny, to 50% results in positive values of 0.75 and 0.90, respectively.

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### 13.1.5 Urinary Cortisol/Creatinine Ratio

The urinary cortisol/creatinine ratio is best used to reduce the probability of a diagnosis of hyperadrenocorticism. Hyperadrenocorticism is very unlikely in a dog with normal urinary cortisol/creatinine ratio. Dogs with hyperadrenocorticism and those with nonadrenal disease often have an increased urinary cortisol/creatinine ratio. In dogs with an increased urinary cortisol/creatinine ratio, the clinical findings may aid

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in the selection of the low-dose dexamethasone suppression test or the ACTH stimulation test as the better choice for additional testing. It is probably better to have the owner collect the urine sample in the dog's home environment to reduce the potential influence of stress. Cortisol and creatinine are measured in the urine and a ratio is calculated. A cortisol/creatinine ratio within the reference range indicates that hypercortisolemia is not present, and therefore hyperadrenocorticism is unlikely the cause of the clinical sign(s).

### 13.1.6 Alkaline Phosphatase Activity

Circulating ALP activity is frequently elevated in dogs with hyperadrenocorticism, making the presence of the disease unlikely in a dog with a value within the reference range. Conversely, an increased serum ALP value commonly occurs in dogs administered exogenous corticosteroids and those with nonadrenal disease. Hepatocellular nodular regeneration is a notably common cause of a raised value in aged dogs with an incidence that increases with age. The determination of the corticosteroid-induced ALP isoenzyme, which is unique to the dog, generally does not add significant diagnostic value. Its sensitivity may be as high as 95% (increased value in dogs with hyperadrenocorticism or treated with corticosteroid medication), but its specificity may be as low as 18% (increased in many dogs without hyperadrenocorticism). If it were measured routinely as part of a biochemical profile, its absence would make a diagnosis of glucocorticoid excess unlikely.

### 13.1.7 Use of the Low-Dose Dexamethasone Suppression Test and Adrenocorticotrophic Hormone Stimulation Test in the Dog

The low-dose dexamethasone suppression test is generally more sensitive but less specific than the ACTH stimulation test. Therefore it is more likely to show lack of suppression (i.e., positive result) in dogs with hyperadrenocorticism but is also associated with a higher incidence of false-positive results when nonadrenal illness is present. A false-positive result is approximately three times as likely for the low-dose dexamethasone suppression test compared with the ACTH stimulation test in dogs with nonadrenal disease. Conversely, the ACTH stimulation test misses approximately 5% to 15% and the low-dose dexamethasone suppression test approximately 5% of dogs with hyperadrenocorticism (i.e., false-negative result; values remain within reference range).

The low-dose dexamethasone suppression test (0.01 to 0.015 mg/kg, administered intravenously) appears to be the better test when the clinical signs are highly compatible with corticosteroid excess and the dog is not currently receiving exogenous corticosteroid or phenobarbital. Pituitary-dependent hyperadrenocorticism is supported by suppression of the serum cortisol concentration at 4 hours (e.g.,  $\leq 1.5 \mu\text{mol/L}$ ;  $\leq 30 \text{ nmol/L}$ ) but not at 8 hours after the administration of dexamethasone or less than a 50% reduction of either or both of the 4- or 8-hour serum cortisol values compared with the predexamethasone (baseline) value.

The ACTH stimulation test appears to be the better initial choice in evaluating the dog with inconclusive or nondescript clinical signs with or without an unexplained moderate to marked increase in the circulating ALP activity or receiving phenobarbital. The ACTH stimulation test is used to monitor the response to medical management of hyperadrenocorticism and to determine whether treatment has resulted in glucocorticoid deficiency (adrenal insufficiency). It is useful for the diagnosis of hypoadrenocorticism and iatrogenic hypoadrenocorticism (clinical signs of hyperadrenocorticism secondary to prolonged glucocorticoid therapy).

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### 13.1.8 Combination Testing

Occasionally a combination testing strategy of a high-dose dexamethasone suppression test (0.1 mg/kg, administered intravenously) and ACTH stimulation test provides additional information in differentiating

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pituitary-dependent and adrenal-dependent hyperadrenocorticism. When a combination testing strategy is used, the dexamethasone suppression test (either low-dose or high-dose) can be conducted the day after the ACTH stimulation test. If the ACTH stimulation test is conducted initially, the low-dose dexamethasone suppression test is performed 2 days later or the high-dose dexamethasone suppression test performed 5 days later.

### 13.1.9 Plasma 17-Hydroxyprogesterone

The measurement of another component of the adrenal steroid production pathway may be diagnostically helpful in dogs with clinical signs and clinicopathologic changes, including raised alanine aminotransferase, alkaline phosphatase, or both enzyme activities, but have negative ACTH stimulation test and low-dose dexamethasone suppression test results. The circulating 17-hydroxyprogesterone concentration samples taken during the ACTH stimulation test may be increased in the post-ACTH plasma ( $>1.32$  ng/mL;  $>4$  nmol/L).

### 13.1.10 Factitious Cortisol Values

High cortisol values will be obtained if certain glucocorticoids (e.g., prednisolone, topical hydrocortisone) have been administered. Dexamethasone does not interfere with the cortisol assay.

#### 13.1.10.1 Feline Hyperadrenocorticism

The dexamethasone suppression test is the test of choice for the diagnosis of hyperadrenocorticism in cats. Dexamethasone is administered intravenously at a dose of 0.1 mg/kg. The serum cortisol concentration is measured before the administration of dexamethasone and at 4 and 8 hours after administration. Approximately 25% of cats with hyperadrenocorticism have false-negative results. Hyperglycemia is a common concurrent finding.

#### 13.1.10.2 Equine Hyperadrenocorticism

Horses with pituitary pars intermedia dysfunction (“equine Cushing’s disease”) have an increased appetite, abnormal hair coats that are thick and wavy (hirsutism), laminitis, polyuria/polydipsia, loss of muscle mass resulting in muscle weakness, raised intermittent hyperpyrexia, somnolence, and excess generalized sweating (hyperhidrosis). Hyperglycemia, often insulin-resistant, can be present. Some of the clinical findings are probably due to the large size of some tumors that cause a functional disturbance of the hypothalamus. Baseline plasma cortisol and ACTH concentrations may be within reference ranges or only slightly elevated but cortisol secretion is not suppressed by dexamethasone. One testing strategy uses an overnight dexamethasone suppression test. Dexamethasone (40 µg/kg or 2 mg/100 lb) is administered intramuscularly between 5 and 6 PM. Plasma is obtained for cortisol measurements before and 19 hours after dexamethasone administration. A plasma cortisol concentration following the administration of dexamethasone that is greater than 1 µg/dL is consistent with the diagnosis. The measurement of cortisol in saliva may provide another approach for assessing adrenal function in horses. Plasma cortisol measurements associated with treadmill testing or subsequent to ACTH stimulation testing may be used as a physiological marker for performance evaluation.

#### 13.1.10.3 Hyperadrenocorticism in Ferrets

In contrast to hypercortisolemia as the prominent biomarker of hyperadrenocorticism in dogs, cats, and horses, the disease in ferrets is diagnosed by finding excessive concentrations of one or more androgens and

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estrogens. These include androstenedione, 17-hydroxyprogesterone, and estradiol. A subset of dogs may display a similar hormonal profile. Anecdotal reports of dogs note clinical signs with or without microscopic changes in the liver suggestive of excess glucocorticoids that have unremarkable cortisol values when tested but have excess sex steroids. The referral laboratory should be contacted for sampling instructions.

### 13.1.10.4 Effect of Portosystemic Shunts on the Hypothalamic-Pituitary-Adrenal Axis in Dogs

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Dogs with encephalopathy secondary to congenital or acquired portosystemic shunts can have a dysfunctional hypothalamic-pituitary-adrenal axis. ACTH and  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ MSH) concentrations may be increased, and the osmotic threshold of vasopressin (antidiuretic hormone) release is abnormally elevated. The latter may be a consequence of prolonged hypercortisolemia secondary to the increased ACTH secretion. The basal circulating cortisol concentration can be increased, and it may rise into the “gray zone” following ACTH stimulation but it does not reliably suppress with the low-dose dexamethasone suppression test. Another abnormality of cortisol metabolism is an increase in the free cortisol concentration in the plasma (the biologically active component) due to a reduction in the circulating cortisol-binding globulin. These changes may contribute in part to the development of the polyuria/polydipsia, increased alkaline phosphatase, and microscopic changes in the liver suggestive of excess circulating glucocorticoids identified in some dogs with portosystemic shunts.

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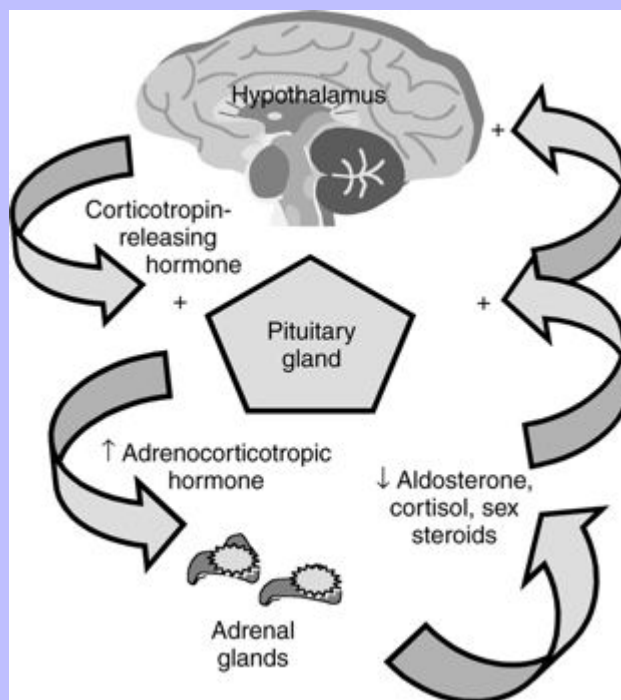
### 13.1.11 Canine Hypoadrenocorticism (Fig. 12-4, Box 12-1, Case 12-1)

Primary hypoadrenocorticism is usually a consequence of bilateral adrenal atrophy in which all three layers of the cortex are markedly reduced in thickness, with attendant deficiency in production of adrenal sex steroid, glucocorticoids, and mineralocorticoids. Secondary hypoadrenocorticism results from a deficiency of CRH or ACTH caused by hypothalamic or pituitary pathology. It is a rare cause of glucocorticoid deficiency. Iatrogenic secondary hypoadrenocorticism is commonly associated with the prolonged use of exogenous glucocorticoids which suppress the hypothalamic-pituitary-adrenal axis.

The deficiency of circulating glucocorticoids and mineralocorticoids often causes a vague history of poor appetite, weakness, trembling, weight loss, vomiting, diarrhea, and regurgitation associated with megaesophagus. Dehydration and depression are often the only findings noted from a physical examination. Occasionally an adrenal “crisis” is manifested by hypotensive shock, collapse, and bradycardia—the latter finding a dichotomy in association with hypotension.

A mild normocytic, normochromic anemia may exist but not be apparent because of concurrent dehydration related to electrolyte and water imbalance. If the dehydration is severe enough, a relative erythrocytosis will be present.

Fig. 12-4



Dysfunctional hypothalamic-pituitary-adrenal axis caused idiopathic adrenocortical atrophy. A marked reduction (loss) of the tissue comprising all three layers of the adrenal cortex of both adrenal glands is present. The resultant deficiency of cortisol and aldosterone production is responsible for the clinical manifestations of hypoadrenocorticism. Concurrent deficient production of the adrenal sex steroids usually occurs. *CRH*, Corticotropin-releasing hormone; *ACTH*, adrenocorticotropic-releasing hormone.

Multiple biochemical abnormalities are present. The lack of mineralcorticoid production often results in hyperkalemia and hyponatremia. A sodium/potassium ratio of less than 27 develops in most dogs with hypoadrenocorticism. The basal cortisol concentration is less than the reference range and does not increase appreciably following the administration of ACTH.

The corticosteroid deficiencies and concurrent water and electrolyte imbalance can result in renal dysfunction suggestive of primary renal disease. The urea nitrogen, creatinine, and phosphorus concentrations are frequently elevated. For reasons that are unclear, the urine specific gravity often does not appropriately reflect the marked dehydration and is less than 1.030, which further suggests primary renal disease and confounds the recognition of hypoadrenocorticism. Because hypoadrenocorticism is most common in young adult to middle-aged dogs, the presence of renal insufficiency in that age group should raise the suspicion of adrenal dysfunction as a potential underlying disease. The possibility of hypoadrenocorticism is heightened if the findings are in breeds with a higher predilection (e.g., standard poodle, Great Dane, rottweiler, Portuguese water spaniel, Wheaten terrier, and West Highland white terrier). The sodium/potassium ratio should be carefully scrutinized, and it is prudent to save serum for a possible cortisol measurement before initiating medical management. In a sick, stressed dog, the cortisol value would be expected to be normal to increased if adrenal function is adequate.

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Other possible but less common biochemical changes include hypercalcemia, a reduction in total carbon dioxide, chloride, and glucose concentrations. Hepatic enzyme activities and bilirubin concentration may be increased. Subclinical hypoadrenocorticism is one cause of a persistent increase in serum aminotransferase activities in human beings.

### 13.1.11.1 BOX 12-1 Causes of Reduced Sodium/Potassium Ratio (<27)<sup>\*</sup>

Hypoadrenocorticism

Renal disease

Repeated drainage of effusions (pleural and peritoneal)

Diarrhea associated with trichuriasis (whipworm infection)

Diabetic ketoacidosis

\* The sodium or potassium value can be within reference range. Generally, the lower the ratio, the more likely hypoadrenocorticism is the cause of the electrolyte abnormality. Because the clinical signs of hypoadrenocorticism are often nonspecific and because of the common development of azotemia secondary to prerenal insufficiency, a misdiagnosis of primary renal disease may initially be made. Measurement of the plasma cortisol concentration before and after adrenocorticotrophic hormone stimulation is prudent in cases with renal insufficiency and an abnormal sodium/potassium ratio, especially in young to middle-aged female adult dogs of a predisposed breed (standard poodle, Great Dane, rottweiler, West Highland white terrier, Wheaton terrier, Leonberger, and Portuguese water dog).

### 13.1.12 Feline Hypoadrenocorticism

Adrenal cortical insufficiency is rare in cats. Clinical signs are similar to those in dogs with the exception of gastrointestinal signs. Lethargy and poor appetite are common, in addition to dehydration that may be detected as prolonged capillary refill time and radiographic microcardia, and indications of reduced pulmonary perfusion. Hematological and biochemical changes are similar to those in dogs, including findings indicative of renal failure. Recurrent renal insufficiency that responds rapidly to medical management should prompt the consideration of hypoadrenocorticism. An abnormal ACTH stimulation test result is diagnostic.

### 13.1.13 Excessive Mineralcorticoid Secretion in Cats and Dogs

Excessive mineralcorticoid secretion is a rare consequence of an adrenal neoplasm. In cats, primary hyperaldosteronism is characterized by hypokalemia, inappropriate kaliuresis, increased circulating aldosterone, and hypertension. Increased urea nitrogen may be present. In the dog, excessive secretion of deoxycorticosterone is associated with metabolic alkalosis, hypokalemia, and hypertension. An enlarged adrenal gland is demonstrable with abdominal ultrasonography.

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### 13.2 THYROID GLAND

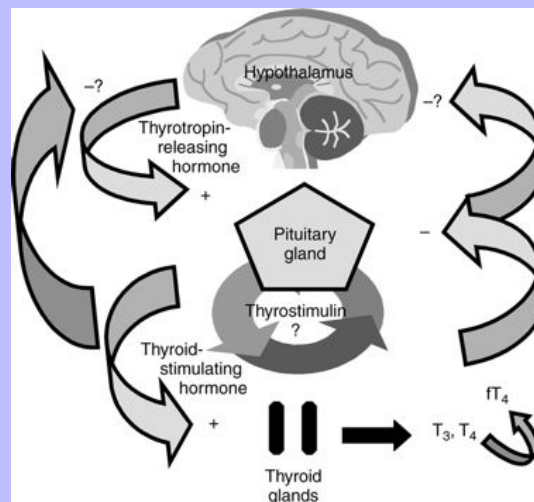
#### 13.2.1 Thyroid Hormones and Their Regulation (Fig. 12-5)

Iodine and tyrosine (part of thyroglobulin) are the predominate substrates in the synthesis of the main thyroid hormones, tetraiodothyronine ( $T_4$ ) and  $T_3$ . The hormones are stored in abundance as a colloid within the acinar lumen. Their release involves a series of steps that translocates them through the follicular cell with cleavage from the thyroglobulin. The majority of  $T_3$  formation results from the subsequent deiodination of  $T_4$  in the skeletal muscle, liver, and kidney. A type of  $T_3$  referred to as reverse  $T_3$  ( $rT_3$ ) is also formed but it has little biologic activity. Thyroxine-binding protein is the primary carrier of  $T_4$  and  $T_3$  in the dog. It is absent in the cat. Albumin and thyroxine-binding prealbumin (transthyretin) also transport thyroid hormones, the latter specific for  $T_4$ . Most of the thyroid hormone is protein bound with only about 0.1% existing as  $fT_4$ , the biologically active component, in the dog. Drugs and endogenous substances easily influence the protein-binding equilibrium (e.g., estrogen shifts it to the bound form). In contrast to most hormones that have circulating plasma half-lives of seconds to minutes,  $T_4$  has a circulating half-life of 6 to 7 days in the dog. This is probably due to the high proportion that is protein bound. In contrast, the circulating plasma half-life in the cat is approximately 6 to 8 hours.

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Fig. 12-5



Hormonal interactions that regulate the hypothalamic-pituitary-thyroid axis. A reduction of free thyroxine ( $fT_4$ ) initiates the release of thyrotropin-releasing hormone (TRH), which stimulates the pituitary gland to release thyroid-stimulating hormone (TSH) into the peripheral circulation. It stimulates the thyroid glands to secrete the thyroid hormones. An increase in the circulating  $fT_4$  level inhibits the release of TSH from the pituitary gland (negative feedback). Thyostimulin is a novel pituitary hormone named for its ability to stimulate thyrotropin receptors. It is a potent stimulator of thyroid cell functions in vitro and in vivo and, although it may have been involved in a paracrine-signaling mechanism, its specific role in thyroid physiology remains to be defined.  $T_3$ , Triiodothyronine.

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Thyroid-stimulating hormone regulates thyroid activity. Its release from the anterior pituitary is facilitated by thyrotropin-releasing hormone (TRH) from the hypothalamus. The release of TSH and TRH is regulated by negative feedback inhibition from the thyroid hormones. Glucocorticoids, androgens, and growth hormone can suppress TSH secretion.

Thyrostimulin is a novel pituitary hormone named for its ability to stimulate thyrotropin (TSH) receptors. It is a potent stimulator of thyroid cell functions in vitro and in vivo and may have a paracrine-signaling function. Its specific physiological role in thyroid hormone homeostasis is unknown but unraveling its contribution will undoubtedly contribute to a better understanding of the complexity of thyroid physiology, pathophysiology, and testing strategies.

## 13.2.2 Canine Hypothyroidism (Fig. 12-6; Case 12-2)

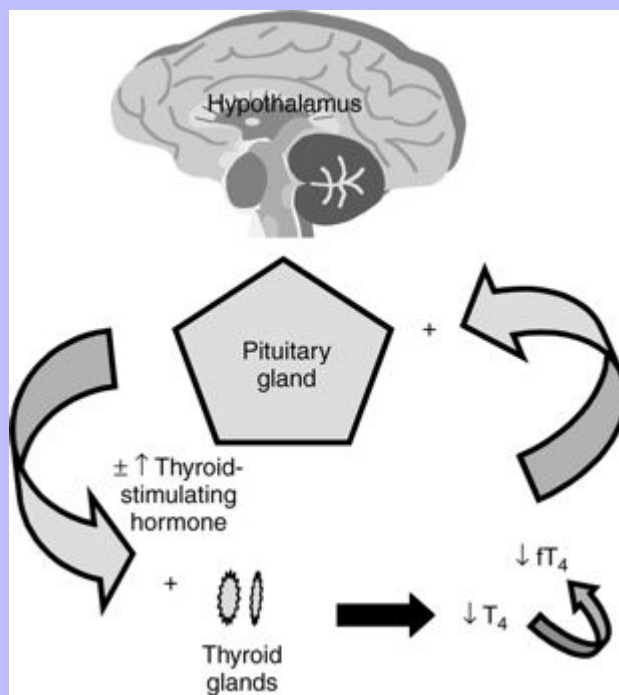
Hypothyroidism in the dog is manifested clinically by lethargy, mental dullness, weight gain, and symmetric endocrine alopecia. Neuromuscular dysfunction and reproductive problems occur less commonly. An uncommon but dramatic clinical presentation is a syndrome composed of myxedema, stupor, and coma. An immune-mediated mechanism is involved in the pathogenesis of hypothyroidism. Other endocrinopathies with a proposed immunopathogenic mechanism, such as diabetes mellitus and primary hypoadrenocorticism, may develop concomitantly.

Diagnosis of hypothyroidism is problematic because of the nondescript clinical signs and the reduction of serum thyroid hormone concentrations secondary to concurrent nonthyroidal disease. A careful history and physical examination are essential to identify underlying disease. The signalment is helpful. A higher incidence of hypothyroidism exists in middle-aged, medium to large breeds, such as golden and Labrador retrievers, Doberman pinscher, Irish setter, miniature schnauzer, and cocker spaniel. Laboratory findings can include a mild normochromic, normocytic, nonregenerative anemia, hypercholesterolemia, and elevated circulating alkaline phosphatase and creatine kinase activities. Hypothyroidism and acquired von Willebrand's disease may coexist because of the incidence of both diseases in the breeds affected.

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Fig. 12-6



Pathology involving both thyroid glands in dogs (idiopathic follicular atrophy or lymphocytic thyroiditis) results in the reduction of circulating thyroid hormones. Although a reduction of the circulating total thyroxine ( $T_4$ ) level occurs in hypothyroidism, it is also reduced in euthyroid dogs secondary to drugs and non-thyroidal disease (“euthyroid sick syndrome”). A total  $T_4$  concentration within reference range is not consistent with hypothyroidism. The free  $T_4$  ( $fT_4$ ) concentration (measured by equilibrium dialysis) is more sensitive and specific for canine hypothyroidism than total  $T_4$  because it is not commonly affected by non-thyroidal disease. Occasionally a total  $T_4$  concentration below the reference range is measured in cats. Because feline primary hypothyroidism is rare, the reduced circulating total  $T_4$  concentration is most likely due to non-thyroidal disease (euthyroid sick syndrome). The  $fT_4$  concentration (measured by equilibrium dialysis) is within reference range if the cat is euthyroid. *TSH*, Thyroid-stimulating hormone.

### 13.2.2.1 Total $T_4$ versus Free $T_4$ versus TSH; $T_3$

The measurement of the serum thyroid hormones should be used to support a high index of clinical suspicion of hypothyroidism and for the assessment of treatment. A normal total  $T_4$  level indicates that hypothyroidism is not a likely diagnosis. A total  $T_4$  value that is below reference range values is problematic since non-thyroidal factors (systemic disease, drugs [e.g., chronic trimethoprim/sulfamethoxazole]) can depress total  $T_4$  values without concurrent clinically important thyroid deficiency or pathologic condition. The  $fT_4$

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level is less commonly reduced than total  $T_4$  secondary to non-thyroidal factors making it a more sensitive and more specific test for diagnosis of hypothyroidism. A  $fT_4$  value within reference range in a dog with a reduced total  $T_4$  level makes the diagnosis of hypothyroidism unlikely. Free  $T_4$  should always be measured by the equilibrium dialysis method. Measurement of  $fT_4$  by this method is valid in dogs with thyroid autoantibodies.

Total  $T_4$  is used to monitor thyroid medication unless autoantibodies are present (value raised above the reference range). A total  $T_4$  value of 2.3 to 4.6  $\mu\text{g/dL}$  (30-60  $\text{nmol/L}$ ) 4 to 8 hours after the administration of the medication is considered appropriate. The administration of thyroid medication will suppress thyroid function in a euthyroid dog within a month. The assessment of thyroid function in a dog receiving thyroid medication is problematic. To reassess or confirm a diagnosis, the thyroid medication is withheld for 1 month followed by measurement of  $fT_4$ . A value within reference range is not consistent with canine hypothyroidism. An additional 1-month withdrawal is conducted if the  $fT_4$  concentration is equivocal.

Circulating TSH concentrations are an enigma in canine hypothyroidism. The loss of negative feedback inhibition should result in increased TSH concentrations. However, it is not increased in approximately one fourth to one third of documented hypothyroid dogs for reasons that are not clear. Its use in thyroid testing strategies should be conducted in concordance with the recommendations of the laboratory performing the assay.

The measurement of circulating  $T_3$  does not have diagnostic value for the diagnosis of canine hypothyroidism. If a  $T_3$  measurement is provided as part of a thyroid panel, an elevated value is suggestive of concurrent autoantibodies; a phenomenon of unknown cause or significance. Although autoantibodies cause increased values for  $T_3$ ,  $T_4$ , or both values by the methodology used, they do not adversely affect the equilibrium dialysis method for the measurement of  $fT_4$ . A reduced  $fT_4$  concentration is indicative of hypothyroidism. A  $fT_4$  concentration within reference range suggests that hypothyroidism is not the cause of the clinical presentation but should be followed every 3 to 6 months.

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### 13.2.2.2 Breed-Related Differences in Normal Values

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The greyhound has lower values than other breeds and could be mistakenly classified as hypothyroid. The laboratory should be contacted for breed-related reference ranges.

### 13.2.3 Feline Hypothyroidism

Occasionally a total  $T_4$  concentration below the reference range is measured in cats. Because feline primary hypothyroidism is rare, the reduced circulating total  $T_4$  concentration is most likely due to non-thyroidal disease ("euthyroid sick syndrome"). The  $fT_4$  concentration (measured by equilibrium dialysis) will be within reference range if primary hypothyroidism is not present.

### 13.2.4 Equine Hypothyroidism

Hypothyroidism affects equine neonates and adult horses. Incoordination, ineffective suckling, and musculoskeletal deformities are manifested in neonates. More characteristic signs of hypothyroidism, such as lethargy, exercise intolerance, and alopecia, occur in adult horses. Hypothyroidism may also contribute to the

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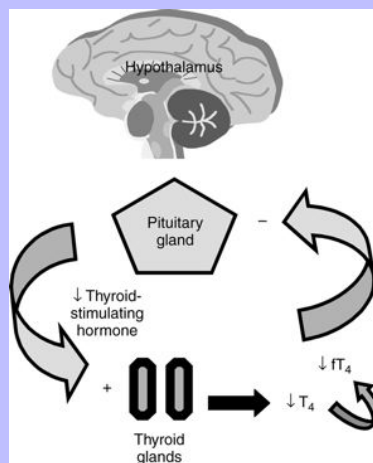
pathophysiology of recurrent laminitis, chronic myositis, and reduced fertility. The use and interpretation of thyroid test results for assessing thyroid function is not well defined. The measurement of equine TSH in conjunction with selected thyroid hormones appears to improve the documentation of hypothyroidism. The appropriate veterinary endocrine laboratory should be contacted for testing strategies.

Thyroid hormones affect lipid metabolism. In canine hypothyroidism, an increase in the circulating cholesterol concentration is a frequent finding. The development of dyslipidemia in the horse may be an indirect indication of hypothyroidism. In an experimental setting subsequent to thyroidectomy, the circulating concentrations of very-low-density (VLDL), low-density lipoproteins (LDL), total cholesterol, and total triglycerides increased within 4 weeks.

### 13.2.5 Feline Hyperthyroidism (Fig. 12-7)

Functional adenomatous hyperplasia (sometimes indicative of adenoma) of the thyroid gland results in feline hyperthyroidism. Generally there is bilateral hyperplasia of thyroid glands. Weight loss, polyphagia, vomiting, polydipsia/polyuria, and hyperactivity are the more common clinical signs. Hematological findings include a high normal or slightly elevated hematocrit and mean cell volume (MCV). Biochemically, one or more of the liver enzyme test results (alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase) are frequently raised. The total bilirubin concentration is increased in some hyperthyroid cats, which in association with abnormal liver enzyme tests, is suggestive of primary cholestatic liver disease. However, there are no to minimal microscopic alterations in liver biopsies suggestive of a functional disturbance. The abnormal liver test results resolve with management of the hyperthyroidism. Raised serum urea nitrogen and creatinine concentrations may be present.

Fig. 12-7



Feline adenomatous hyperplasia (adenoma), involving both thyroid glands in approximately 75% of cats, results in increased circulating thyroid hormone levels. Sometimes the total thyroxine ( $T_4$ ) concentration is in the upper end of the reference range in a cat with clinical signs consistent with hyperthyroidism. The test can be repeated at a later date because the total  $T_4$  concentration fluctuates in mild cases, or the free  $T_4$  ( $fT_4$ ) concentration can be measured and will be elevated if the disease is present. *TSH*, Thyroid-stimulating hormone.

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A raised circulating total  $T_4$  concentration is diagnostic in most cats with hyperthyroidism. Cats with clinical signs consistent with hyperthyroidism but a total  $T_4$  concentration in the upper end of the reference range should be considered to have hyperthyroidism that is mild, or the total  $T_4$  concentration may be suppressed by a concurrent non-thyroidal disease. The total  $T_4$  concentration occasionally fluctuates above the reference range on retesting, which confirms the diagnosis. Similar to dogs, the circulating  $fT_4$ , measured by equilibrium dialysis, is often representative of thyroid function in the presence of non-thyroidal factors disease. The caveat is that it may be increased in some euthyroid cats with non-thyroid disease. Measuring both the total  $T_4$  and  $fT_4$  is helpful. A total  $T_4$  value in the upper end of the reference and a raised  $fT_4$  concentration is indicative of hyperthyroidism. A total  $T_4$  value in the lower end of the reference and a raised  $fT_4$  concentration is suggestive of a euthyroid cat with a concurrent non-thyroidal disease. A  $T_3$  suppression test or thyrotropin-releasing hormone stimulation test can be conducted if additional testing is needed. Testing strategies and evaluation of the thyroid values should be conducted in concordance with the recommendations of the laboratory performing the assay. Measurement of the total  $T_4$  concentration is used to assess medical management. The value should be in the lower end of the reference range.

## 13.3 PARATHYROID GLAND

### 13.3.1 Bone and Mineral Metabolism (Figs. 12-8 and 12-9)

The orchestration of calcium homeostasis is mediated through the integrated actions of parathyroid hormone (PTH), vitamin D, and calcitonin. The bone, the small intestine, and the kidney are the three principal target organs. Estrogens, glucocorticoids, somatotropin, glucagon, and  $T_4$  can influence calcium metabolism but their adverse effects are not a common clinical concern in veterinary medicine.

The majority of the body calcium, phosphate, and magnesium are in the skeleton. The hydroxyapatite crystal,  $Ca_{10}(PO_4)_6(OH)_2$ , contributes to the mechanical strength of bone and serves as a reservoir for calcium and phosphate. The concentrations of these minerals in the plasma and extracellular fluid are controlled within narrow limits. Approximately 50% of the ionized calcium ( $Ca^{2+}$ ) is bound to albumin. Albumin has approximately 12 calcium-binding sites per molecule with only approximately 20% occupied normally. Physiological changes can rapidly affect calcium binding (e.g., an increase or reduction in blood pH increases or decreases, respectively, the binding affinity). A small quantity of calcium forms complexes with anions. Approximately 70% of the circulating phosphate is bound to phospholipids and phosphoproteins with the remainder as inorganic phosphate. The majority circulates as ions or complexes of  $HPO_4^{2-}$  and  $H_2PO_4^-$ . One point of clarification regarding phosphate/phosphorus terminology is necessary; only phosphate circulates in blood and is the constituent that is measured, not elemental phosphorus per se. Ideally, it would be biochemically more appropriate to report values as phosphate expressed as milligrams per deciliter of phosphorus or as millimoles per liter of phosphate in SI units. However, the term *phosphorus* has been traditionally used to indicate the measurement concentration of circulating phosphate.

#### 13.3.1.1 Circulating Total Calcium and Albumin Relationship

The total serum calcium concentration can be “adjusted” for changes in the plasma albumin concentration using the following formula, validated for dogs.

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Adjusted calcium (mg/dL) =  
measured calcium (mg/dL) – albumin (g/dL) + 3.5

The bone, kidney, and small intestine are the key organs involved in phosphate homeostasis. Dietary intake is the main source of phosphate. The intestinal absorption of phosphate is enhanced by vitamin D and growth hormone. In the growing animal, the high level of growth hormone is one cause of the increased circulating phosphate concentration. Most of the phosphate in circulation undergoes glomerular filtration with the majority reabsorbed by the renal tubules. Parathyroid hormone inhibits that tubular function resulting in phosphaturia.

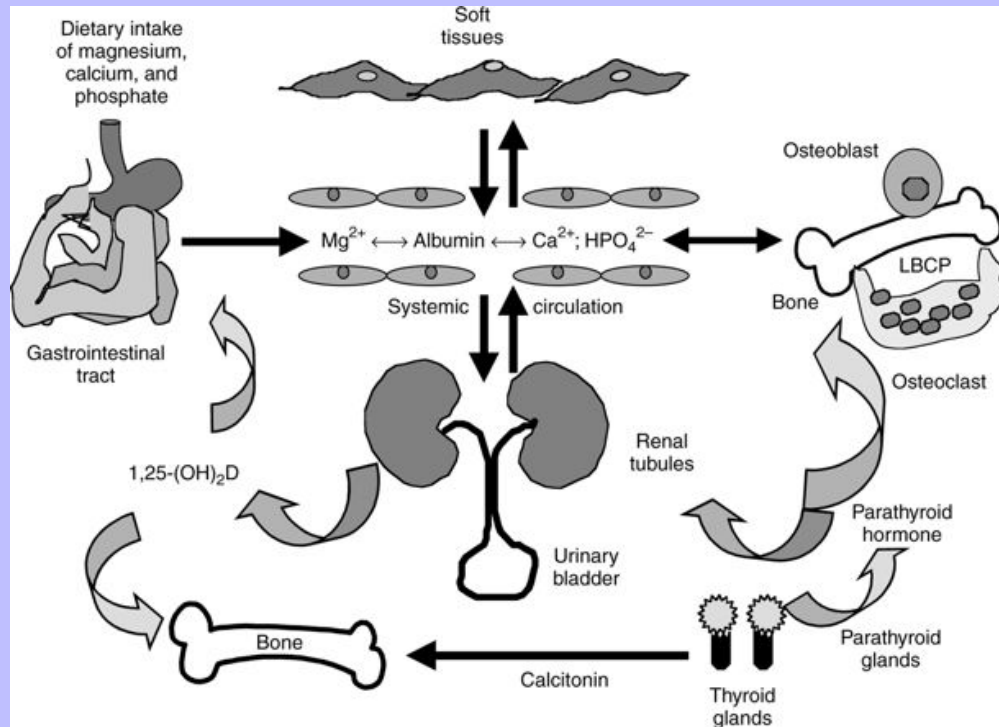
### 13.3.2 Magnesium Metabolism (see [Fig. 12-8](#))

Magnesium is ingested in the diet, with grasses and green plants an excellent source for ruminants. As a consequence, magnesium-related clinical disorders were initially recognized in ruminants. Hypomagnesemia also contributes to clinical findings in dogs and cats. It is the second most abundant cation in soft tissues (potassium is the most common). Because of the prominent tissue reservoir, measurement of circulating magnesium, either total or ionized, may not accurately reflect the magnesium status in relationship to the clinical condition. Measurement of its concentration in peripheral blood mononuclear cells (PBMCs) may be a better reflection of the magnesium state. Magnesium is stored in bone but unlike calcium, it is not readily mobilized in deficient states. Magnesium uses the calcium-binding sites on albumin but a lower affinity compared with calcium results in only about 25% being albumin bound. In contrast to calcium, approximately 30% of the extracellular magnesium complexes with anions (phosphate, sulfate, citrate). As an intracellular catalyst of many reactions, magnesium plays a prominent role in muscle contraction, cell and mitochondrial membrane stability, and oxidative phosphorylation. It intimately interacts with calcium in many cell functions and influences the regulation of intracellular potassium concentration.

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Fig. 12-8



Parathyroid hormone (PTH), vitamin D<sub>3</sub> (cholecalciferol, calcitriol), and calcitonin (CT) are the hormones that regulate the circulating calcium concentration and bone metabolism. Parathyroid hormone is considered to be the major physiological regulator for maintenance of normal circulating calcium concentrations. Calcitonin appears to play more of an emergency backup role in response to abrupt rises in the plasma calcium concentrations. Change in the circulating ionized calcium concentration is the predominant signal for modification of the secretion rates of these hormones. Calcitonin is secreted by C cells (parafollicular) in the thyroid glands, distinct from the follicular cells that synthesize and secrete thyroid hormones, in response to an increase in the circulating ionized calcium concentration and a reduction in the circulating ionized calcium concentration inhibits calcitonin secretion (negative feedback loop). Calcitonin impairs the release of ionized calcium from the labile bone calcium pool (LBCP), a resorption pit formed by the catabolic action of the osteoclast, by inhibition of PTH-stimulated bone resorption mediated by a complex osteoblast-osteoclast interaction.

Chief cells of the parathyroid glands secrete parathyroid hormone. A reduction in the circulating ionized calcium concentration stimulates the calcium-sensing receptor on the parathyroid cell to increase PTH secretion, whereas an increase in the circulating calcium concentrations inhibits PTH secretion (negative feedback loop). There is both an immediate PTH response to the change in circulating calcium concentrations and a slower response (hours to days) that is mediated by changes in PTH messenger RNA. Parathyroid hormone modulates release of calcium from the LBCP, the acidic microenvironment of bone degradation sandwiched between the surface of the bone matrix and the ruffled membrane of the osteoclast. The PTH effect on the osteoclast is indirect because it has no PTH receptors. Through

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complex and incompletely understood paracrine factors that involve a critical osteoblast-osteoclast interaction, PTH affects bone calcium metabolism by activating osteoblasts. Parathyroid hormone modulates circulating calcium and phosphate concentrations through effects on renal tubular function. It causes retention of calcium by stimulating its reabsorption by the distal convoluted tubule and reduces the circulating phosphate concentration by directly impairing its reabsorption by the proximal tubule of the nephron resulting in phosphaturia. Parathyroid hormone secretion can be indirectly inhibited by vitamin D<sub>3</sub> metabolites through activation of vitamin D receptors on the parathyroid cell to decrease PTH messenger RNA synthesis.

Vitamin D<sub>3</sub> (cholecalciferol) modulates circulating calcium and phosphate concentrations predominantly through effects on the intestine. Dietary cholecalciferol or that formed in the skin is converted to 25-hydroxycholecalciferol (25-hydroxyvitamin D, calcidiol) in the liver and transported to the kidney where 1,25-dihydroxycholecalciferol (1,25-(OH)<sub>2</sub>D, calcitriol, 1,25-dihydroxyvitamin D) is formed by the action of 1- $\alpha$ -hydroxylase, the rate-limiting step in vitamin D metabolism. Circulating parathyroid hormone and phosphate concentrations modulate formation of 1,25-dihydroxycholecalciferol. A reduction of the circulating phosphate concentration stimulates and a rise of the circulating phosphate concentration inhibits 1- $\alpha$ -hydroxylase activity resulting in either an increase or decrease, respectively, in the circulating level of 1,25-dihydroxycholecalciferol. An increase in the circulating 1,25-dihydroxycholecalciferol level has a negative feedback effect on 1- $\alpha$ -hydroxylase activity. Parathyroid hormone is the more potent stimulant of the formation of 1,25-dihydroxycholecalciferol, which in turn has a negative feedback effect that causes inhibition of PTH synthesis by modulating PTH gene transcription. The biologically active form of vitamin D<sub>3</sub>, 1,25-dihydroxycholecalciferol, increases the absorption of calcium and phosphate in the proximal and distal segments of the small intestine, respectively. Vitamin D<sub>3</sub> metabolites enhance PTH-mediated renal tubular reabsorption of calcium. Vitamin D<sub>3</sub> metabolites are potent promoters of osteoclastic bone resorption that becomes readily available at bone surfaces for mineralization of the osteoid matrix formed by osteoblasts.

### Key Biological Actions

#### *PTH*

- Stimulates osteoclast-mediated bone resorption and release of calcium and phosphate from bone
- Stimulates renal tubular reabsorption of calcium and inhibits renal tubular reabsorption of phosphate
- Stimulates renal formation of 1,25-dihydroxycholecalciferol

#### *Vitamin D<sub>3</sub> (1,25-dihydroxycholecalciferol)*

- Stimulates intestinal absorption of calcium and phosphate
- Stimulates bone resorption
- Enhances the effect of PTH on renal tubular retention of calcium

#### *Calcitonin*

- Inhibits osteoclastic bone resorption—probably minimal impact on long-term calcium homeostasis

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## Summation of Calcium Homeostasis

A reduction in circulating ionized calcium concentration prompts immediate secretion of parathyroid hormone, which increases available calcium to the circulation by stimulation of osteoclastic bone resorption, increased renal tubular reabsorption of calcium. The increased PTH concentration stimulates 1,25-dihydroxycholecalciferol renal synthesis and secretion, which causes increased intestinal absorption of calcium. Conversely, an increase in circulating ionized calcium concentration inhibits PTH secretion, which precludes its calcium-raising influence on bone resorption, renal tubular calcium reabsorption, and intestinal calcium absorption via 1,25-dihydroxycholecalciferol. Bone is a dynamic tissue that is continually remodeling. Bone marrow-derived monocytes are recruited as precursors of osteoclasts that attach to bone surface. Osteoclasts are activated by a complex interaction that involves binding of PTH to osteoblasts that secrete a number of soluble substances that increase osteoclast activity. It responds by sealing the space beneath the cell with a ruffled border. Into this newly created microenvironment it secretes hydrogen ions, lactate, and proteolytic enzymes that are responsible for the breakdown of the protein matrix. The labile bone calcium pool (LBCP) releases ionized calcium and other bone mineral constituents into the systemic circulation. Thus the PTH-mediated catabolic action of osteoclast on bone is an indirect, complex process. Osteoblasts differentiate from connective tissue precursors lining the bone surface. The osteoblast fills the lacuna with osteoid (protein matrix) followed by its mineralization that completes the process of bone formation.

The homeostatic mechanisms regulating magnesium homeostasis are not well understood. 1,25-Dihydroxycholecalciferol facilitates intestinal absorption. An increase in dietary calcium or phosphate reduces intestinal absorption of magnesium. Mineralocorticoids indirectly alter circulating magnesium via effects on sodium and potassium. Hypoaldosteronism is associated with an increase in circulating magnesium and an increase in aldosterone has the opposite effect. Following intestinal absorption it is excreted in the urine. Measurement in urine provides a means of assessing magnesium following supplementation. The mechanism by which hypomagnesemia predisposes to hypocalcemia is not clearly defined. The altered metabolism likely involves an inhibition of PTH secretion and increased tissue resistance to the hormone when circulating magnesium is reduced.

### 13.3.3 Disorders of Mineral Metabolism (Figs. 12-10 to 12-12; Boxes 12-2 to 12-6)

Causes of hypercalcemia, hypocalcemia, hyperphosphatemia, hypophosphatemia, and hypomagnesemia are provided as differential diagnostic considerations. More detail can be obtained in the appropriate textbooks or in the Additional Reading List when more information regarding a particular disorder is necessary. The lists are not prioritized for prevalence of occurrence. However, selected diseases are highlighted. These include primary hyperparathyroidism, humoral hypercalcemia of malignancy, and secondary renal hyperparathyroidism.

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## 13.4 ENDOCRINE PANCREAS

### 13.4.1 Carbohydrate Metabolism

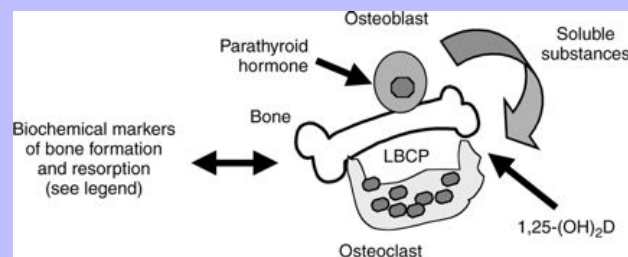
#### 13.4.1.1 Insulin and Glucagon

The endocrine pancreas is composed of microscopic clusters of cells located in the interstitial tissue of the pancreas. These aggregates of hormone-secreting cells are referred to as the *islets of Langerhans*. The islets

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consist of main cell types. The beta cell produces insulin, the alpha cell secretes glucagons, the delta cells contain somatostatin, and PP cells contain pancreatic polypeptide. Insulin promotes glucose utilization, protein synthesis, and formation and storage of lipids. The circulating glucose level primarily regulates the secretion of insulin and glucagons. A reduction of the circulating glucose level inhibits insulin secretion, whereas an increase quickly prompts insulin secretion. The circulating glucose level has the opposite effect on the secretion of glucagon. Glucagon activates hepatic glycogenolysis, which raises the glucose level in the circulation. Growth hormone, glucocorticoids, estrogen, and progesterone increase peripheral resistance to the action of insulin, resulting in accelerated secretion by the beta cells. Somatostatin suppresses the release of insulin and glucagons. Pancreatic polypeptide stimulates the secretion of gastric and intestinal enzymes and has an inhibitory effect on intestinal motility.

Fig. 12-9



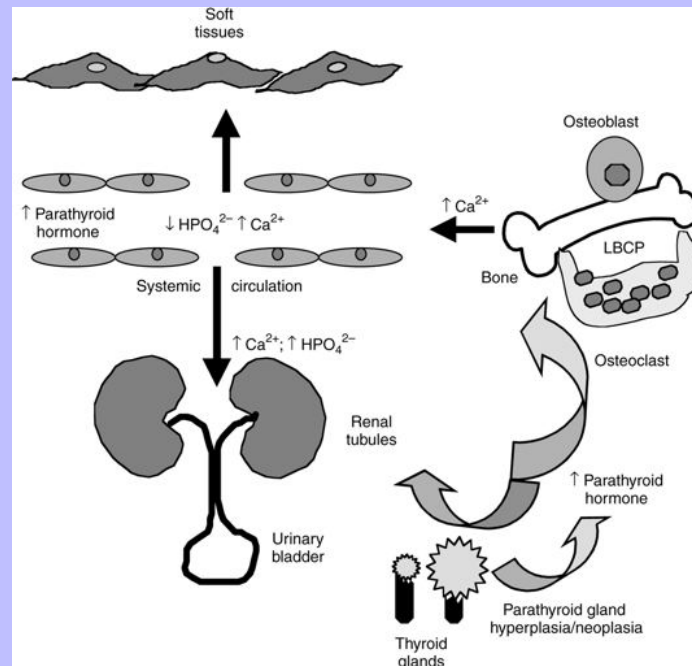
The formation and resorption of bone are coupled processes. Within a given bone metabolic unit (basic multicellular unit), bone resorption is a faster process than is bone formation. In humans for example, the process of bone resorption at a particular site takes 1 to 3 weeks while bone formation takes 2 to 3 months. Consequently, the biochemical markers of bone resorption respond quicker to adverse effects that affect bone. The biochemical markers that reflect bone metabolism are classified as cell-secreted enzymes or proteins, breakdown products subsequent to bone resorption, and by-products generated from the synthesis of new bone. The breakdown products of bone resorption can be measured in the urine or serum. Substances measured in the urine include calcium and components of collagen—hydroxyproline, pyridinoline, deoxypyridinoline, N-telopeptides, and C-telopeptides. Tartrate-resistant acid phosphatase (TRAP) is a measurable lysosomal enzyme in the circulation that is derived from bone. The biochemical markers of bone formation include the measurement of circulating bone isoenzyme of alkaline phosphatase (B-ALP), osteocalcin, a noncollagen protein of bone matrix, and carboxyterminal cross-linked telopeptide of type-1 collagen (ICTP), a collagen breakdown product. Osteoblasts are rich in the bone isoenzyme of alkaline phosphatase and the increased bone activity during growth is the reason for the higher circulating total alkaline phosphatase activity in immature animals. The measurement of bone-related biochemical markers has clinical utility. They are used to follow bone remodeling in lactating dairy cows and study musculoskeletal diseases in dogs and in horses during race training to determine the effects of glucosamine administration. Change in the bone-alkaline phosphatase in dogs with appendicular osteosarcoma before and after surgery has prognostic importance. The measurement of bone-regulating hormones and bone markers can complement the direct assessment of bone mineral density (BMD) determined by use of dual-energy x-ray absorptiometry (DEXA scan). This noninvasive procedure combined with the measurement of serum 1,25-dihydroxycholecalciferol (1,25-(OH)<sub>2</sub>D) and phosphorus concentrations identified season-related reductions of these measurements in alpacas. Alterations in mineral metabolism may predispose growing alpacas to rickets and long bone fractures in adults not exposed to sunlight for prolonged periods. *PTH*, Parathyroid hormone.

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### 13.4.1.2 Hyperglycemia (Fig. 12-13, Box 12-7, Case 11-1)

Insulin facilitates the metabolism of glucose by muscle, adipose tissue, and liver. Insulin deficiency, in concert with continued glucagon activity, results in hyperglycemia and glucosuria. Hyperglycemia caused by the loss of insulin-secreting pancreatic beta cells is referred to as *insulin-dependent diabetes mellitus* (type 1). Non-insulin-dependent diabetes mellitus (type 2) is due to inappropriate insulin secretion by the pancreatic beta cells, with or without peripheral insulin resistance. Weight loss despite polyphagia is a common clinical observation because tissues are effectively starved and compensatory catabolism of fat and muscle accentuates the weight loss. These compensatory inefficient metabolic processes eventually result in ketoacidosis. At this point, the hyperglycemic patient may have a ketotic breath odor and become inappetent and lethargic or depressed. Samoyeds, miniature schnauzers, miniature poodles, pugs, and toy poodles reportedly have an increased risk of developing diabetes mellitus.

Fig. 12-10



Primary hyperparathyroidism. The hyperplastic parathyroid gland tissue autonomously secretes parathyroid hormone (PTH) and is unresponsive to negative feedback suppression. Bone and kidney are the principal organs adversely affected by long-term exposure to increased PTH concentrations. Accelerated bone resorption releases increased calcium and phosphate into the circulation and the phosphaturia is accentuated resulting in hypercalcemia (with or without paradoxical decreased fractional excretion), hypophosphatemia, hypercalciuria, and hyperphosphaturia. The calcium released from bone overwhelms the expected hypocalciuria as a result of PTH-mediated increased renal tubular reabsorption of calcium. Chronic hypercalciuria is nephrotoxic and can result in an early clinical sign of polyuria related to tubular dysfunction. If not treated, progressive nephrotoxicity culminates in renal failure.

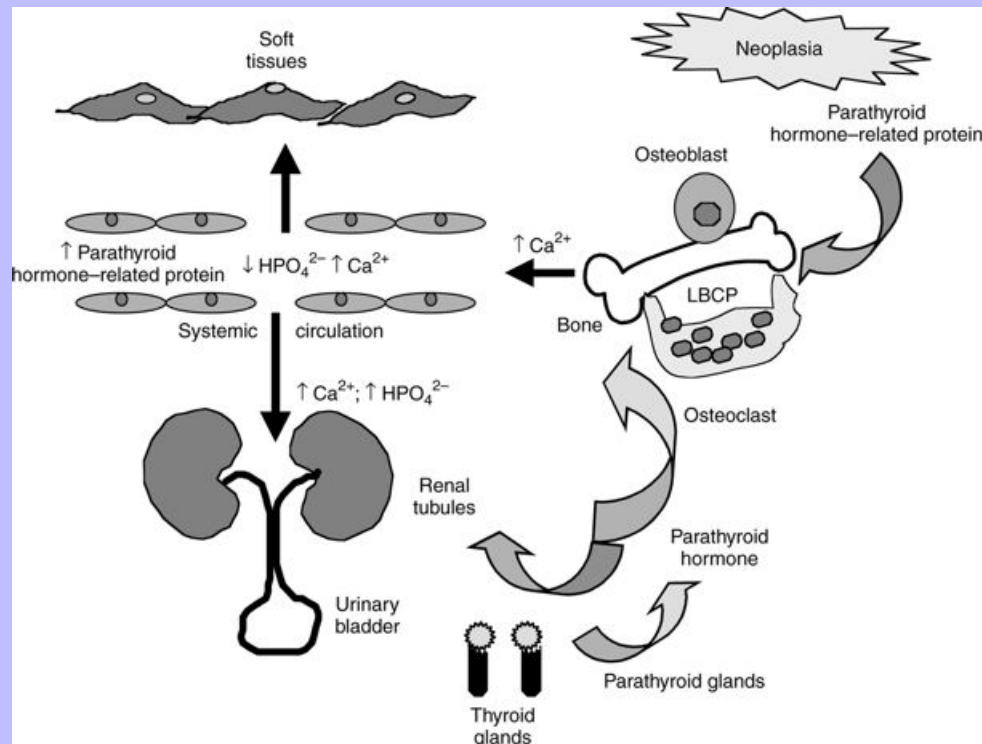
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Growth hormone excess causes acromegaly that initially may be suspected because of hyperglycemia related to its insulin antagonism. In the cat, a pituitary tumor is generally the cause of the excess circulating growth hormone that often results in insulin-resistant diabetes mellitus. Excess circulating growth hormone in female dogs is usually due to excess secretion by the mammary gland when stimulated by progesterone during metestrus or by use of progestogens for preventing estrus.

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Fig. 12-11



The events associated with humoral hypercalcemia of malignancy are illustrated. Parathyroid hormone – related protein is normally formed in multiple tissues and is involved in the local regulation of those tissue functions (autocrine, paracrine). It is not involved in calcium homeostasis. It is so named because it was identified as a product of certain cancers that caused hypercalcemia via affinity for the PTH receptor. It is one important factor associated with humoral hypercalcemia of malignancy. The hypercalcemic syndrome is most commonly associated with lymphoma and carcinomas (n.b., apocrine gland adenocarcinoma of the anal sac in dogs and squamous cell carcinoma in cats), but it can be associated with a variety of neoplasms. The pathophysiological consequences are similar to those associated with primary hyperparathyroidism except that the circulating PTH concentration in concert with the hypercalcemia is normal to reduced and parathyroid-related protein concentration is detected in the circulation. *LBCP*, Labile bone calcium pool; *PTH*, parathyroid hormone.

Additional laboratory findings in insulin-dependent diabetes mellitus may include fasting lipemia, hypercholesterolemia, and ketosis/ketosuria as a result of accelerated lipolysis and increased alkaline phosphatase and alanine aminotransferase activities as a consequence of hepatic lipidosis.

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Challenging the function of the pancreatic beta cell by use of a glucose tolerance test may be diagnostically helpful to uncover latent diabetes mellitus when a persistent, mild hyperglycemia is identified. Several protocols are available and an appropriate reference or laboratory should be use for guidance.

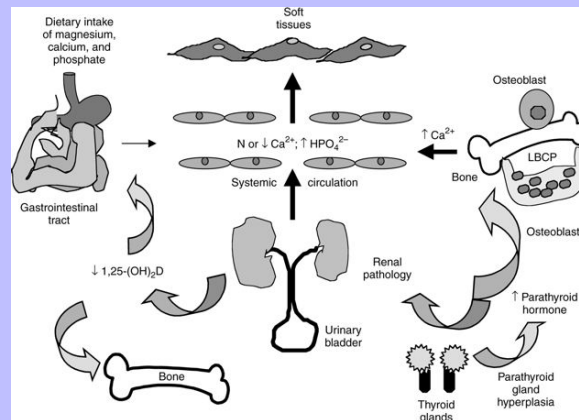
### 13.4.1.3 Glycated Hemoglobin, Fructosamine

Glycated proteins are formed by the nonenzymatic addition of a sugar residue to amino groups. Measurement of glycated hemoglobin is useful for the long-term evaluation of glucose control in dogs and cats. Formation of glycated hemoglobin is irreversible with the circulating level dependent on the erythrocytic life span (approximately 100 days in the dog and 70 days in the cat) and the blood glucose concentration. Consequently, the glycated hemoglobin concentration reflects the circulating glucose level for the preceding several weeks with the more recent glucose level contributing more to its formation than earlier levels. It is not affected by occasional fluctuations related to recent food ingestion, exercise, or stress.

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Fig. 12-12



Events associated with secondary renal hyperparathyroidism. Chronic renal insufficiency results in retention of phosphate (hyperphosphatemia). The increased circulating phosphate level inhibits 1- $\alpha$ -hydroxylase activity, resulting in a reduction of the circulating 1,25-dihydroxycholecalciferol level. Its synthesis is further impaired by a reduction of functional tissue as a result of the renal pathology. A loss of the negative feedback effect on the parathyroid glands occurs due to the reduced circulating 1,25-dihydroxycholecalciferol level. The result is parathyroid gland hyperplasia and progressive increased secretion of PTH secretion with progression of the renal insufficiency. The progressive increase in circulating phosphate causes a reciprocal reduction of circulating calcium that is accentuated by reduced 1,25-dihydroxycholecalciferol-stimulated intestinal absorption of calcium. The chronically increased parathyroid hormone concentration maintains normocalcemia (N) for a period of time at the expense of accelerated bone resorption. A notable clinical finding in the dog is the rubber jaw-like pliability of the mandible and maxilla due to the suppleness of the remaining demineralized organic matrix.

Hypercalcemia is a common biochemical sequela to renal disease in the horse. The circulating phosphate in the horse with renal disease may be reduced or within reference range. Hypercalcemia may develop in cattle and dogs in the late stage of chronic renal disease.

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The glycation of proteins other than hemoglobin results in the formation of protein ketoamines; those in the circulation are referred to with the generic rubric of *fructosamine*. The circulating fructosamine level is dependent on the half-lives of the proteins and the blood glucose concentration. The predominant glycosylated protein contributing to the fructosamine level is considered to be albumin with a circulating half-life of approximately 8 days in the dog and likely of similar or slightly shorter turnover in the cat. Consequently, the fructosamine concentration approximates the average circulating glucose concentration over the preceding 4 to 7 days. It is not meaningfully affected by occasional abrupt fluctuations as a result of recent food ingestion, exercise, or stress (n.b. stress hyperglycemia in cats). Reduced fructosamine levels are reported in hyperthyroid cats, sheep with pregnancy toxemia, ponies with enteric protein loss, and dogs with hypoalbuminemia. Fructosamine is best measured in a sample obtained the same day or stored at  $-20^{\circ}\text{C}$  until measured.

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## 13.4.1.3.1 BOX 12-2 Disorders Causing Hypercalcemia

Hyperalbuminemia (dehydration)

Humoral hypercalcemia of malignancy

Primary hyperparathyroidism

Hypoadrenocorticism

Vitamin D intoxication

Renal disease (horse and cow, uncommon in dog)

Osteolytic bone lesions (e.g., septic osteomyelitis, myeloma)

Plant toxicity (jasmine in dogs and cats; *Cestrum* sp. and *Solanum* sp. [nightshade] in herbivores)

Calciferol-containing rodenticides

Granulomatous diseases with excessive 1,25-dihydroxycholecalciferol production

Hematological malignancies with excessive 1,25-dihydroxycholecalciferol production

Idiopathic—cats

## 13.4.1.3.2 BOX 12-3 Disorders Causing Hypocalcemia

Hypoalbuminemia

Alkalosis (especially in ruminants)

Primary hypoparathyroidism

Renal secondary hyperparathyroidism

Ethylene glycol toxicity (dogs and cats)

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Pancreatitis

Dietary imbalance (hypovitaminosis D, excess phosphorus)

Eclampsia (bitch, mare, ewe) or parturient paresis—"milk fever" (cow)

Intestinal malabsorption/protein-losing enteropathy

Blister beetle poisoning in horses

Hypercalcitonism

Factitious (EDTA used for sample collection)

Iatrogenic, after thyroidectomy (bilateral)

Bicarbonate treatment for salicylate toxicity

Strenuous exercise (equine endurance ride)

Enterocolitis—horses

Massive transfusion

Idiopathic in foals

EDTA, *Ethylenediaminetetraacetic acid*.

### 13.4.1.3.3

#### BOX 12-4 Disorders Causing Hyperphosphatemia

Reduced glomerular filtration rate (renal, prerenal, or postrenal azotemia from any cause)

Factitious; sample held too long before analysis (phosphorus released from erythrocytes)

Growing animals

Dietary phosphorus excess

Phosphorus enema or administration of phosphorus-containing fluids

Hypervitaminosis D

Osteolytic bone disease (neoplasia)

Jasmine toxicity

Massive cell lysis (chemotherapy, rhabdomyolysis)

Hypoparathyroidism with normal glomerular filtration

Hypercalcemia of malignancy with normal glomerular filtration

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Hyperthyroidism in casts without renal insufficiency

Slight increases occasionally from drug treatment (anabolic steroids, furosemide, minocycline, hydrochlorothiazide)

Ethylene glycol toxicity (abrupt increase)

Hypersomatotropism

### 13.4.1.3.4 BOX 12-5 Disorders Causing Hypophosphatemia

Primary hyperparathyroidism (early stages before renal calcinosis)

Hypercalcemia of neoplasia (early stages before renal calcinosis)

Lack of dietary calcium

Hypovitaminosis D

Dogs and cats with diabetes mellitus and ketoacidosis

Respiratory alkalosis related to hyperventilation

Hyperadrenocorticism (about one third of dogs with disorder)

Eclampsia

Hypomagnesemic tetany of ruminants

Parturient paresis in cattle

Malabsorption or starvation

Canine Fanconi-like syndrome

Chronic renal failure in horse

After insulin administration in diabetics

Enteral alimentation

Pseudohyperphosphatemia (associated with myeloma paraprotein)

### 13.4.1.3.5 BOX 12-6 Disorders Causing Hypomagnesemia

Critical illness in dogs and cats

Strenuous exercise (equine endurance ride)

Calves (fed whole milk)

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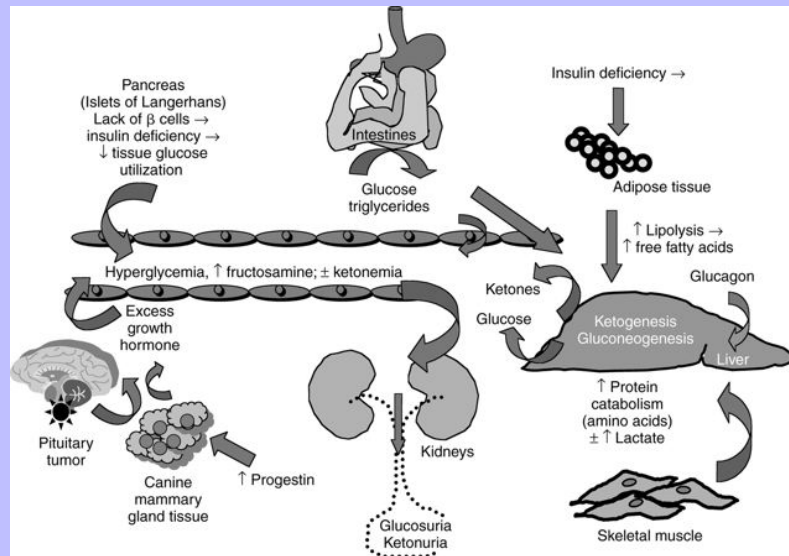
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Grass tetany—adult cattle (initiating spring grass feeding)

Adult sheep and goats (pasture-related)

Transport tetany (lambs)

Fig. 12-13



Events associated with the development of hyperglycemia. Insulin is synthesized in pancreatic beta cells as proinsulin with subsequent cleavage of the C-peptide resulting in insulin. The circulating glucose concentration regulates insulin secretion. The activation of insulin secretion involves the phosphorylation of glucose by glucokinase. Oral glucose prompts greater insulin levels than intravenous administration due to the additional release of gastrointestinal peptides that include glucagons, such as peptide 1 and gastric inhibitory polypeptide. Amino acids and vagal stimulation stimulate insulin secretion. Insulin secretion abruptly declines with the declination of circulating glucose concentration.

Insulin deficiency (type 1 diabetes mellitus) results in the (1) mobilization of substrates for gluconeogenesis and, if severe, ketogenesis from skeletal muscle and adipose tissue, (2) accelerated production of glucose and ketones by the liver, and (3) inefficient uptake of glucose by insulin-dependent tissues. In type 2 diabetes mellitus, resistance of insulin-responsive tissues with or without altered beta cell function results in hyperglycemia. The nonenzymatic addition of a sugar to circulating proteins (glycation) results in the formation of protein ketoamines (fructosamine). The circulating fructosamine level approximates the average circulating glucose level over the preceding 4 to 7 days in dogs and cats.

Counterregulatory hormones antagonize the metabolic actions of insulin. Glucagon is secreted by the pancreatic alpha cells in response to a reduction of the circulating glucose levels and certain amino acids. It stimulates hepatic gluconeogenesis, glycogenesis, and ketogenesis through pathways mediated by cyclic adenosine monophosphate. An increase in the circulating glucose concentration normally inhibits glucagons secretion. This negative feedback pathway may be dysfunctional in diabetes mellitus. The anterior pituitary secretes growth hormone. It inhibits glucose consumption by peripheral tissues and promotes lipolysis. *Lower left*, Excess secretion of growth hormone by a pituitary tumor, notably in cats, or by mammary tissue of female dogs under the influence of progestins causes hyperglycemia.

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## 13.4.1.4 $\beta$ -Hydroxybutyrate

Diabetic ketoacidosis is a life-threatening metabolic complication of untreated diabetes mellitus. The prolonged absence of circulating insulin and actions of the counterregulatory hormones actuate the formation of ketone bodies, acetoacetate and  $\beta$ -hydroxybutyrate. Although increased levels of both ketone bodies indicate the presence of ketosis, an increase in the latter is a more accurate indication of ketoacidosis. The urine reagent strip test (nitroprusside reaction) detects acetoacetate but does not react with  $\beta$ -hydroxybutyrate. A circulating  $\beta$ -hydroxybutyrate concentration of 3.8 mmol/L or greater has been shown to be a useful diagnostic value for the detection of ketoacidosis in diabetic dogs.

### 13.4.1.4.1 BOX 12-7 Causes of Hyperglycemia

Postprandial hyperglycemia (monogastric animals)

Diabetes mellitus

Exertion/stress (epinephrine-associated, more frequent in cats)

Increased glucocorticoids (hyperadrenocorticism, exogenous administration)

Growth hormone excess (acromegaly), especially cats; female dogs with progestins influence)

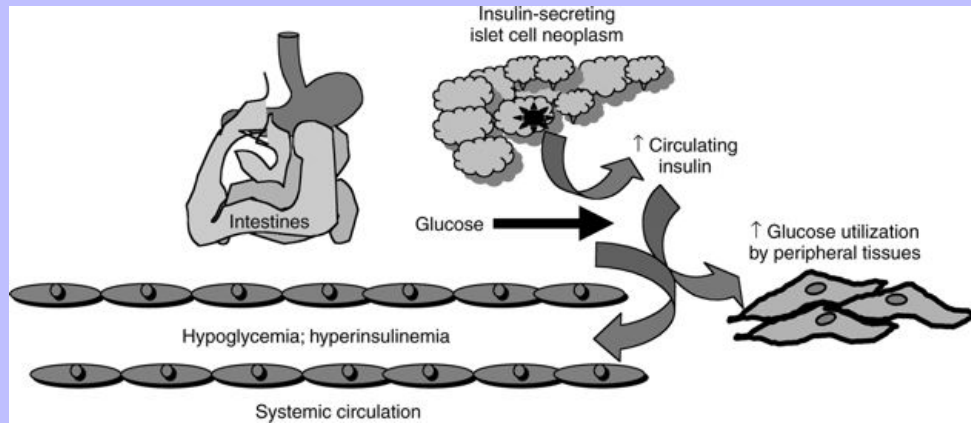
Acute pancreatitis

Drug-induced hyperglycemia (thiazide diuretics, morphine, intravenous fluids with glucose, megestrol acetate, progestins)

Renal insufficiency

Pheochromocytoma

Fig. 12-14



Events associated with development of hypoglycemia. Despite its usual diminutive size, the insulin-secreting islet cell tumor (insulinoma) has a powerful metabolic effect resulting in profound hypoglycemia. Most insulinomas are malignant and metastasis to the liver is common by the time clinical signs of hypoglycemia are observed. Metastases are often functional and secrete insulin, which enhances the hypoglycemia and confounds management. The diagnosis is based on an inappropriate relationship between the circulating glucose and insulin levels (e.g., hypoglycemia [ $<60$  mg/dL]) and normal or increased insulin concentration ( $>10$   $\mu$  U/mL).

## 13.4.2 Hypoglycemia (Fig. 12-14, Box 12-8)

Sample management is critical when hypoglycemia is identified. Glucose utilization by erythrocytes in a blood sample before separation (greater than 30 minutes) or shipment of the serum specimen in a tube that is contaminated by bacteria will result in a hypoglycemic value.

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### 13.4.2.1 BOX 12-8 Causes of Hypoglycemia

- Insulin-secreting (islet cell) neoplasm
- Extrapaneatic neoplasia (primary liver or leiomyosarcoma, hemangiosarcoma)
- Insulin or sulfonylurea therapy
- Delayed separation of serum from erythrocytes
- Congenital portosystemic shunts
- Septicemia/endotoxemia
- Hepatozoon americanum* infection in dogs
- Idiopathic—juvenile in toy breeds, puppies, hunting dogs

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Endocrine hypofunction (hypopituitarism, hypoadrenocorticism)

Canine renal glucosuria (severe cases)

Hepatic glycogen enzyme deficiency (storage disease)

Intermittent—glycogen branching enzyme deficiency in quarterhorse foals

Hepatic insufficiency, severe, acute fulminate or cirrhosis

Chronic renal insufficiency

Prolonged starvation

Fig. 12-15

Physiochemical characteristics of lipoproteins in dogs						
Variable	Chylomicron	VLDL*	LDL	HDL <sub>1</sub> (HDL <sub>c</sub> )	HDL <sub>2</sub> *	HDL <sub>3</sub>
Major apoproteins	B <sub>48</sub>	B <sub>100</sub> , B <sub>48</sub> , E, C	B <sub>100</sub> , B <sub>48</sub>	E, A, C	A, C, E	A, C
Density (g/dL)	<0.930	0.093-1.006	1.019-1.087	1.025-1.100	1.063-1.100	1.100-1.210
Electrophoretic mobility	Origin	pre-β	β	α <sub>2</sub>	α <sub>1</sub>	α <sub>1</sub>
Major lipids	Exogenous triglycerides	Endogenous triglycerides	Cholesterol	Cholesterol	Cholesterol	Cholesterol

\*Predominant canine plasma lipoprotein; transports approximately 80% of the total cholesterol in plasma.

The predominant apoproteins, solvent density, and electrophoretic migration for canine lipoproteins are listed. The four main classes of lipoproteins in domestic animals and rodents have general characteristics similar to those in humans and consist of very-low-density lipoprotein (*VLDL*), low-density lipoprotein (*LDL*), and high-density lipoprotein (*HDL*). The dog, cat, horse, ruminant, and rodents are considered HDL-predominant species. HDL-predominant species are generally resistant to atherosclerosis. Humans, most nonhuman primates, pigs, and rabbits are LDL-predominant species. Dogs have approximately five to six times as much high-density lipoproteins (*HDL*<sub>2</sub>) as lower density lipoproteins. HDL<sub>1</sub> is a unique lipoprotein in dogs that is normally present in very small amounts. Thyroidectomized dogs fed a high cholesterol diet developed markedly increased levels of this cholesterol-enriched high-density lipoprotein (HDL<sub>1</sub>), initially referred to as HDL<sub>c</sub>, and an increase in LDL levels. Some of the dogs developed atherosclerosis. HDL<sub>1</sub> may be erroneously measured as LDL by some analytical methods.

The endogenous production of excess insulin by a functional tumor of the pancreatic beta cells (islet cell tumor) is a common differential consideration for hypoglycemia. The insidious onset of the disease causes intermittent periods of episodic lethargy, weakness, and collapse depending on the fed/fasted state and extent of the pathology. A low/low normal fasted glucose value with a concomitant high normal/increased insulin value supports the diagnosis of an insulin-secreting tumor. Because seizures, collapse, or both are associated with

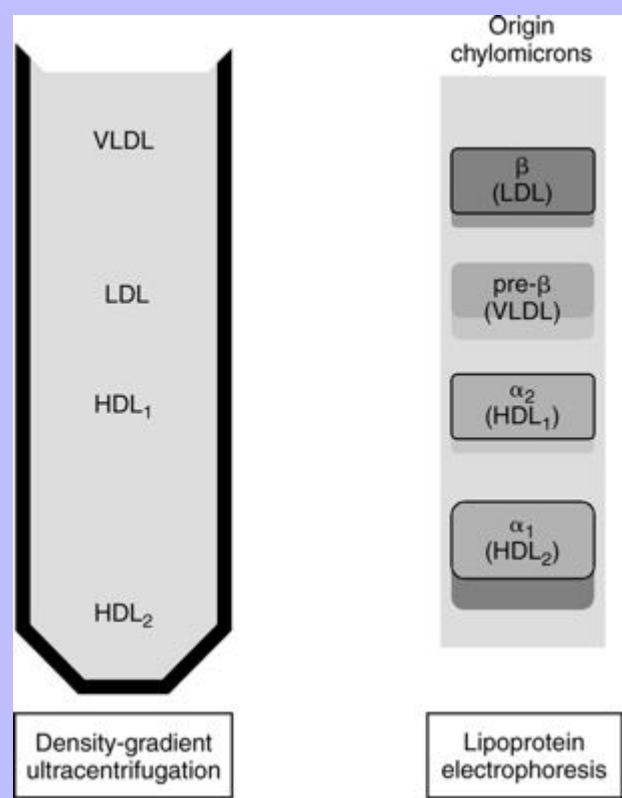
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hypoglycemia, fasting should be judiciously conducted. If the history suggests hypoglycemic events but the glucose value is low normal and the insulin value is within the reference range, the animal is fasted and the glucose monitored hourly. When the blood glucose value is approximately 40 mg/dL, serum is obtained for a concomitant insulin measurement and frequent small meals are fed.

Insulin/glucose and amended insulin/glucose ratios have been evaluated for enhancing the biochemical diagnosis of insulin-secreting tumors. In general, they provide no additional diagnostic assistance. The foundation for the evaluation of suspected islet cell insulin-secreting hypoglycemia is based on the concomitant relationship of the circulating insulin and glucose concentrations, historical and physical findings, assessment of the other organ systems with hematological and clinical chemistry profiles, and abdominal ultrasonography.

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Fig. 12-16



The relationship of canine plasma lipoproteins determined by density-gradient ultracentrifugation and lipoprotein electrophoresis. *VLDL*, Very-low-density lipoprotein, low-density lipoprotein, *LDL*, *HDL*<sub>1</sub>, high-density lipoprotein 1, *HDL*<sub>2</sub>, high-density lipoprotein 2.

## 13.4.2.2 BOX 12-9 Causes of Hypercholesterolemia/Hyperlipidemia

### 13.4.2.2.1 Secondary to Metabolic Disorder/Disease

Postprandial (hypertriglyceridemia prominent)

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13.4.2.2.2

Hypothyroidism (hypercholesterolemia most common in dogs)

Diabetes mellitus

Hyperadrenocorticism

Protein-losing nephropathy (nephrotic syndrome)

Pancreatitis

Chronic cholestatic liver disease or extrahepatic bile duct obstruction

**Primary Hyperlipidemia**

Idiopathic hyperlipoproteinemia (n.b., miniature schnauzer)

Idiopathic hyperchylomicronemia (n.b., cat)

Lipoprotein lipase (n.b., cat)

Fasting equine hyperlipidemia (n.b., ponies)

13.4.2.3

## BOX 12-10 Causes of Hypocholesterolemia

Congenital portosystemic vascular anomaly

Maldigestion/exocrine pancreatic insufficiency

Malabsorption/protein-losing enteropathy/lymphangiectasia

Severe malnutrition

13.5

## LIPID METABOLISM (Figs. 12-15, 12-16; Boxes 12-9, 12-10)

Lipoproteins are lipid-protein complexes that transport triglycerides and cholesterol from sites of origin in the intestine and the liver to tissues for energy storage and cell utilization. Four major classes of lipoproteins are defined by particle size, chemical composition, flotation characteristics, and electrophoretic migration. They are chylomicrons, VLDLs, LDLs, and high-density lipoprotein (HDL). The chylomicron is the relatively large lipoprotein fraction that causes the postprandial milky, turbid appearance of plasma/serum. After digestion of triglycerides, the monoglycerides and fatty acids pass into the intestinal epithelial cells, which resynthesizes triglycerides. The triglyceride-rich fat droplets enter the intestinal lymph as chylomicrons and are transported through the thoracic duct and emptied into circulation at the juncture of the jugular and subclavian veins. They are the least dense of the lipoproteins and will float to the top of a refrigerated plasma/serum specimen.

In contrast to human beings, the assessment of the lipoprotein levels in domestic animals has relatively limited importance because of the rarity of atherosclerotic disease. Human beings have a predominant LDL metabolism that readily reacts to fat and cholesterol consumption, resulting in lipid deposition in the intima of large and medium-sized arteries and provoking inflammation, fibrosis, and calcification. The altered lipoprotein profiles for various endocrine disorders have been variably characterized in dogs, cats, and horses but their clinical diagnostic

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utility is generally limited by the relatively complex methodology and the availability of better testing strategies. The routine total cholesterol and triglyceride measurements are generally adequate biochemical markers of dyslipidemia that can be pursued by the appropriate endocrine testing strategy when abnormal results are found. Characterization of altered lipoprotein metabolism may be of value in clinical research to define disorders that lack a more specific test. For example, determining the lipoprotein profile in the horse may be helpful in identifying and characterizing dyslipidemia associated with hypothyroidism. After thyroidectomy in horses, the circulating concentrations of VLDL, LDL, total cholesterol, and total triglycerides increased within 4 weeks. Lipoprotein profiles are also used to characterize metabolic alterations in disease states such as canine lymphoma.

### 13.6 ADDITIONAL READING\*

#### 13.6.1 Parathyroid (Calcium and Phosphorus) and Magnesium Metabolism

- \*MJ Allen: Biochemical markers of bone metabolism in animals: uses and limitations. *Vet Clin Pathol.* **32**, 2003, 101–113.
- \*MJ Allen, WE Hoffmann, DC Richardson, et al.: Serum markers of bone metabolism in dogs. *Am J Vet Res.* **59**, 1998, 250–254.
- BT Altura, JL Burack, RQ Cracco, et al.: Clinical studies with the NOVA ISE for  $\text{I Mg}^{2+}$ . *Scand J Clin Lab Invest.* **54**, 1994, 53–67.
- \*S Bateman: Cats and magnesium—Another species to consider [editorial]. *J Vet Intern Med.* **16**, 2002, 215–216.
- \*AP Bolliger, PA Graham, V Richard, et al.: Detection of parathyroid hormone-related protein in cats with humoral hypercalcemia of malignancy. *Vet Clin Pathol.* **31**, 2002, 3–8.
- \*JP Caron, TL Peters, JG Hauptman, et al.: Serum concentrations of keratin sulfate, osteocalcin, and pyridinoline crosslinks after oral administration of glucosamine to standardbred horses during race training. *Am J Vet Res.* **63**, 2002, 1106–1110.
- TL Clemens, JA Fagin: Parathyroid hormone-related protein: New insights into its normal biology. *Endocrine.* **2**, 1994, 871–873.
- AJ Dart, JR Snyder, SJ Spier, et al.: Ionized calcium concentration in horses with surgically managed gastrointestinal disease: 147 cases (1988–1990). *J Am Vet Med Assoc.* **201**, 1992, 1244–1248.
- \*MB Demay, MS Kiernan, HF DeLuca, et al.: Sequences in the human parathyroid gene that binds the 1,25-dihydroxyvitamin D<sub>3</sub>. *Proc Natl Acad Sci USA.* **89**, 1992, 8097–8101.
- RJ Elin: Magnesium: The fifth but forgotten electrolyte. *Clin Chem.* **102**, 1994, 616–622.
- DC Ferguson: Update on diagnosis of canine hypothyroidism. *Vet Clin North Am.* **24**, 1994, 515–540.
- \*N Ehrhart, WS Dernell, WE Hoffmann, et al.: Prognostic importance of alkaline phosphatase activity in serum from dogs with appendicular osteosarcoma: 75 cases (1990–1996). *J Am Vet Med Assoc.* **213**, 1998, 1002–1006.
- BJ Gerloff, EP Swenson: Acute recumbency and marginal phosphorus deficiency in dairy cattle. *J Am Vet Med Assoc.* **208**, 1996, 716–719.
- TK Graves, ME Peterson: Diagnostic tests for feline hyperthyroidism. *Vet Clin North Am.* **24**, 1994, 567–576.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

A Jaggy, JE Oliver, DC Ferguson, et al.: Neurological manifestations of hypothyroidism: A retrospective study of 29 dogs. *J Vet Intern Med.* **8**, 1994, 328–336.

\*C Khanna, EM Lund, M Raffae, et al.: Hypomagnesemia in 188 dogs: A hospital population-based prevalence study. *J Vet Intern Med.* **12**, 1998, 304–309.

AT Kristensen, JS Klausner, DJ Weiss, et al.: Spurious hyperphosphatemia in a dog with chronic lymphocytic leukemia and an IgM monoclonal gammopathy. *Vet Clin Pathol.* **20**, 1991, 45–48.

\*A Liesegang, R Eicher, ML Sassi, et al.: Biochemical markers of bone formation and resorption around parturition and during lactation in dairy cows with high and low standard milk yields. *J Dairy Sci.* **83**, 2000, 1773–1781.

\*A Liesegang, R Reutter, ML Sassi, et al.: Diurnal variation in concentration of various markers of bone metabolism in dogs. *Am J Vet Res.* **60**, 1999, 949–953.

\*SJ Marx: Hyperparathyroidism and hypoparathyroid disorders. *N Engl J Med.* **343**, 2000, 1863–1875.

MA McLoughlin, AP DiBartola, SJ Birchard, et al.: Influence of systemic nonthyroidal illness on serum concentration of thyroxine in hyperthyroid cats. *J Am Anim Hosp Assoc.* **29**, 1993, 227–234.

NT Messer, PJ Johnson, KR Refsal, et al.: Effect of food deprivation on baseline iodothyronine and cortisol concentrations in healthy, adult horses. *Am J Vet Res.* **56**, 1995, 116–121.

HP Meyer, J Rothuizen: Increased free cortisol in plasma of dogs with portosystemic encephalopathy (PSE). *Domest Anim Endocrinol.* **11**, 1994, 317–322.

\*AM Midkiff, DJ Chew, JF Randolph, et al.: Idiopathic hypercalcemia in cats. *J Vet Intern Med.* **14**, 2000, 619–626.

R Moreau, RA Squires: Hypercalcemia. *Comp Cont in Educ Pract Vet.* **14**, 1992, 1077–1087.

\*GR Mundy, TA Guise: Hormonal control of calcium homeostasis. *Clin Chem.* **45**, 1999, 1347–1352.

S Oren, A Feldman, S Turkot, et al.: Hyperphosphatemia in multiple myeloma. *Ann Hematol.* **69**, 1994, 41–43.

\*JE Parker, KI Timm, BB Smith, et al.: Seasonal interaction of serum vitamin D concentration and bone density in alpacas. *Am J Vet Res.* **63**, 2002, 948–953.

JT Potts: Non-traditional actions of parathyroid hormone: An overview. *Miner Electrolyte Metab.* **21**, 1995, 9–12.

\*BM Pressler, DS Rotstein, JM Law, et al.: Hypercalcemia and high parathyroid hormone-related protein concentration associated with malignant melanoma in a dog. *J Am Vet Med Assoc.* **221**, 2002, 263–265.

\*LG Raisz: Physiology and pathophysiology of bone remodeling. *Clin Chem.* **45**, 1999, 1353–1358.

J Rothuizen, WJ Biewenga, JA Mol: Chronic glucocorticoid excess and impaired osmoregulation of vasopressin release in dogs with hepatic encephalopathy. *Domest Anim Endocrinol.* **12**, 1995, 13–24.

\*J Silver, T Naveh-Many, H Mayer, et al.: Regulation by vitamin D metabolites of parathyroid hormone gene transcription in vivo in the rat. *J Clin Invest.* **78**, 1986, 1296–1301.

\*GJ Stewler: The physiology of parathyroid hormone-related protein. *N Engl J Med.* **342**, 2000, 177–185.

\*SL Teitelbaum: Bone resorption by osteoclasts. *Science.* **289**, 2000, 1504–1508.

\*J Toll, H Erb, N Birnbaum, T Schermerhorn: Prevalence and incidence of serum magnesium abnormalities in hospitalized cats. *J Vet Intern med.* 2002, 221, 16217.

CB Waters, CR Scott-Moncrieff: Hypocalcemia in cats. *Comp Contin Educ Pract Vet.* **4**, 1992, 497–505.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

\*NB Watts: Clinical utility of biochemical markers of bone remodeling. *Clin Chem.* **45**, 1999, 1359–1368.

\*ID Wijnberg, JH van der Kolk, H Franssen, et al.: Electromyographic changes of motor unit activity in horses with induced hypocalcemia and hypomagnesemia. *Am J Vet Res.* **63**, 2002, 849–856.

M Zaidi, VS Shankar, LH Huang, et al.: Molecular mechanisms of calcitonin action. *Endocrine.* **2**, 1994, 459–467.

## 13.6.2 Thyroid

\*BA Breuhaus: Thyroid-stimulating hormone in adult euthyroid and hypothyroid horses. *J Vet Intern Med.* **16**, 2002, 109–115.

JD Broussard, ME Peterson, PR Fox: Changes in clinical and laboratory findings in cats with hyperthyroidism from 1983-1993. *J Am Vet Med Assoc.* **206**, 1995, 302–305.

LA Frank: Comparison of thyrotropin-releasing hormone (TRH) to thyrotropin (TSH) stimulation for evaluating thyroid function in dogs. *J Am Anim Hosp Assoc.* **32**, 1996, 481–487.

\*N Frank, JE Sojka, MA Latour, et al.: Effect of hypothyroidism on blood lipid concentrations in horses. *Am J Vet Res.* **60**, 1999, 730–733.

\*KR Gaughan, DS Bruyette: Thyroid function testing in greyhounds. *Am J Vet Res.* **62**, 2001, 1130–1133.

IA Hall, KL Campbell, MD Chambers, et al.: Effect of trimethoprim/sulfamethoxazole on thyroid function in dogs with pyoderma. *J Am Vet Med Assoc.* **202**, 1993, 1959–1962.

\*LB Kantrowitz, ME Petersen, C Melian, R Nichols: Serum total thyroxine, total triiodothyronine, free thyroxine, and thyrotropin concentrations in dogs with nonthyroidal disease. *J Am Vet Med Assoc.* **219**, 2001, 765–769.

EM Kaptein, GE Moore, DC Ferguson, et al.: Effects of prednisolone on thyroxine and 3,5,3'-triiodothyronine metabolism in normal dogs. *Endocrinology.* **130**, 1992, 1669–1679.

RJ Kempainen, DW Young, EN Behrend, et al.: Autoantibodies to triiodothyroxine and thyroxine in a golden retriever. *J Am Anim Hosp Assoc.* **32**, 1996, 195–198.

MG Larsson: Determination of free thyroxine and cholesterol as a new screening test for canine hypothyroidism. *J Am Anim Hosp Assoc.* **24**, 1988, 209–217.

\*CT Mooney, CL Little, AW Macrae: Effect of illness not associated with the thyroid gland on serum total and free thyroxine concentrations in cats. *J Am Vet Med Assoc.* **208**, 1996, 2004–2008.

K Muth Beale, Halliwell, REW, CL Chen: Prevalence of antithyroglobulin antibodies detected by enzyme-linked immunosorbent assay of canine serum. *J Am Vet Med Assoc.* **196**, 1990, 745–748.

\*K Nakabayashi, H Matsumi, A Bhalla, et al.: Thyrostimulin, a heterodimer of two new human glycoprotein hormone subunits, activates the thyroid-stimulating hormone receptor. *J Clin Invest.* **109**, 2002, 1445–1452.

\*DL Panciera: Editorial: Thyroid function tests—what do they really tell us?. *J Vet Intern Med.* **15**, 2001, 86–88.

DL Panciera: Hypothyroidism in dogs: 66 cases (1987-1992). *J Am Vet Med Assoc.* **204**, 1994, 761–767.

DL Panciera, GS Johnson: Plasma von Willibrand factor antigen concentration and buccal mucosal bleeding time in dogs with experimental hypothyroidism. *J Vet Intern Med.* **10**, 1996, 60–64.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

- \*ME Peterson, C Melian, CR Nichols: Measurement of serum total thyroxine, triiodothyronine, and thyrotropin concentrations for diagnosis of hypothyroidism in dogs. *J Am Vet Med Assoc.* **211**, 1997, 1396–1402.
- ME Peterson, JD Broussard, DA Gamble: Use of the thyrotropin releasing hormone stimulation test to diagnose mild hyperthyroidism in cats. *J Vet Intern Med.* **8**, 1994, 279–286.
- ME Peterson, TK Grave, DA Gamble: Triiodothyroxine (T<sub>3</sub>) suppression test. *J Vet Intern Med.* **4**, 1990, 233–238.
- EL Thacker, KR Refsal, RW Bull: Prevalence of autoanti-bodies to thyroglobulin, thyroxine, or triiodothyronine and relationship of autoantibodies and serum concentrations of iodothyronines in dogs. *Am J Vet Res.* **53**, 1992, 449–453.
- DA Williams, C Scott-Moncrieff, J Bruner, et al.: Validation of an immunoassay for canine thyroid-stimulating hormone and changes in serum concentration following induction of hypothyroidism in dogs. *J Am Vet Med Assoc.* **209**, 1996, 1730–1732.
- \*FE Wondisford: The thyroid axis just got more complicated. *J Clin Invest.* **109**, 2002, 1401–1402.
- M Paradis, N Page: Serum free thyroxine concentrations measured using chemiluminescence in hyperthyroid and euthyroid cats. *J Am Anim Hosp Assoc.* **32**, 1996, 489–494.

### 13.6.3 Adrenal Glands

- \*EN Behrend, RJ Kemppainen: Glucocorticoid therapy: pharmacology, indications, and complications. *Vet Clin North Am Small Anim Pract.* **27**, 1999, 187–213.
- \*EN Behrend, RJ Kemppainen: Diagnosis of canine hyperadrenocorticism. *Vet Clin North Am Small Anim Pract.* **31**, 2001, 985–1003.
- \*EN Behrend, RJ Kemppainen, TP Clark, et al.: Diagnosis of hyperadrenocorticism in dogs: a survey of internists and dermatologists. *J Am Vet Med Assoc.* **220**, 2002, 1643–1649.
- R Boulton, MI Hamilton, AP Dhillon, et al.: Subclinical Addison's disease: A cause of persistent abnormalities in transaminase values. *Gastroenterology.* **109**, 1995, 1324–1327.
- L Couetil, MR Paradis, J Knoll: Plasma adrenocorticotropin concentration in healthy horses and in horses with clinical signs of hyperadrenocorticism. *J Vet Intern Med.* **10**, 1995, 1–6.
- NO Dybdal, KM Hargreaves, JE Madigan, et al.: Diagnostic testing for pituitary pars intermedia dysfunction in horses. *J Am Vet Med Assoc.* **204**, 1994, 627–632.
- EC Feldman, RW Nelson, MS Feldman: Use of the low- and high-dose dexamethasone tests for distinguishing pituitary-dependent from adrenal tumor hyperadrenocorticism in dogs. *J Am Vet Med Assoc.* **209**, 1996, 772–775.
- \*SM Flood, JF Randolph, Gelzer, ARM, et al.: Primary hyperaldosteronism in two cats. *J Am Hosp Assoc.* **35**, 1999, 411–416.
- \*LA Frank, LP Schmeitzel, JW Oliver: Steroidogenic response of adrenal tissues after administration of ACTH to dogs with hypercortisolemia. *J Am Vet Med Assoc.* **218**, 2001, 214–216.
- Goossens, MMC, HP Meyer, G Voorhout, et al.: Urinary excretion of glucocorticoids in the diagnosis of hyperadrenocorticism in cats. *Domest Anim Endocrinol.* **12**, 1995, 355–362.
- \*DS Greco, ME Peterson, AP Davidson, et al.: Concurrent pituitary and adrenal tumors in dogs with hyperadrenocorticism—17 cases (1978-1995). *J Am Vet Med Assoc.* **214**, 1999, 1349–1353.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- AM Grooters, DS Biller, SK Theisen, et al.: Ultrasonographic characteristics of the adrenal glands in dogs with pituitary-dependent hyperadrenocorticism: Comparison with normal dogs. *J Vet Intern Med.* **10**, 1996, 110–115.
- CJ Henry, TP Clark, DW Young, et al.: Urine cortisol: creatinine ratio in healthy and sick cats. *J Vet Intern Med.* **10**, 1996, 123–126.
- AJ Kaplan, ME Peterson, RJ Kemppainen: Effects of disease on the results of diagnostic tests for use in detecting hyperadrenocorticism in dogs. *J Am Vet Med Assoc.* **207**, 1995, 445–451.
- SJ Lifton, LG King, CA Zerbe: Glucocorticoid deficient hypoadrenocorticism in dogs: 18 cases (1986-1995). *J Am Vet Med Assoc.* **209**, 1996, 2076–2081.
- RE Mack: Screening tests used in the diagnosis of canine hyperadrenocorticism. *Semin Vet Med Surg.* **9**, 1994, 118–122.
- \*M Marc, N Parvizi, F Ellendorff, et al.: Plasma cortisol and ACTH concentrations in the warmblood horse in response to a standardized treadmill test as physiological markers for evaluation of training status. *J Anim Sci.* **78**, 2000, 1936–1946.
- ME Peterson, DS Greco, DN Orth: Primary hypoadrenocorticism in ten cats. *J Vet Intern Med.* **3**, 1989, 55–58.
- ME Peterson, RJ Kemppainen: Dose-response relation between plasma concentrations of corticotropin and cortisol after administration of incremental doses of cosyntropin for corticotropin stimulation testing in cats. *Am J Vet Res.* **54**, 1993, 300–304.
- ME Peterson, RJ Kemppainen, DN Orth: Effects of synthetic ovine corticotropin-releasing hormone on plasma concentrations of immunoreactive adrenocorticotropin, alpha-melanocyte-stimulating hormone, and cortisol in dogs with naturally acquired adrenocortical insufficiency. *Am J Vet Res.* **53**, 1992, 421–425.
- ME Peterson, PP Kintzer, PH Kass: Pretreatment clinical and laboratory findings in dogs with hypoadrenocorticism: 225 cases (1979-1993). *J Am Vet Med Assoc.* **208**, 1996, 85–91.
- \*JE Prittie, LJ Barton, ME Peterson, et al.: Pituitary ACTH and adrenocortical secretion in critically ill dogs. *J Am Vet Med Assoc.* **220**, 2002, 615–619.
- \*Ristic, JME, IK Ramsey, FM Heath, et al.: The use of 17-hydroxyprogesterone in the diagnosis of canine hyperadrenocorticism. *J Vet Intern Med.* **16**, 2002, 433–439.
- KL Rosenthal, ME Peterson: Evaluation of plasma androgen and estrogen concentrations in ferrets with hyperadrenocorticism. *J Am Vet Med Assoc.* **209**, 1996, 1097–1102.
- D Sadek, M Schaer: Atypical Addison's disease in the dog: A retrospective survey of 14 cases. *J Am Anim Hosp Assoc.* **32**, 1996, 159–163.
- \*LP Schmeitzel, CD Lothrop: Hormonal abnormalities in Pomeranians with normal coat and in Pomeranians with growth hormone-response dermatosis. *J Am Vet Med Assoc.* **197**, 1990, 1333–1341.
- LE Smiley, ME Peterson: Evaluation of a urine cortisol: creatinine ratio as a screening test for hyperadrenocorticism in dogs. *J Vet Intern Med.* **7**, 1993, 163–168.
- \*HM Syme, JC Scott-Moncrieff, NG Treadwell, et al.: Hyperadrenocorticism associated with excess sex hormone production by an adrenocortical tumor in two dogs. *J Am Vet Med Assoc.* **219**, 2001, 1725–1728.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

\*CH Van Liew, DS Greco, MD Salman: Comparison of results for adrenocorticotrophic hormone stimulation and low-dose dexamethasone suppression test with necropsy findings in dogs: 81 cases (1985-1995). *J Am Vet Med Assoc.* **211**, 1997, 322–325.

\*JH Van der Kolk, RF Nachreiner, HC Schott, et al.: Salivary and plasma concentration of cortisol in normal horses and horses with Cushing's disease. *Equine Vet J.* **33**, 2001, 211–213.

## 13.6.4 Endocrine Pancreas

\*DJ Appleton, JS Rand, J Priest, et al.: Determination of reference values for glucose tolerance, insulin tolerance, and insulin sensitivity tests in clinically normal cats. *Am J Vet Res.* **62**, 2001, 630–636.

\*EN Behrend, DS Greco: Feline diabetes mellitus: Evaluation of treatment. *Compend Contin Educ Pract Vet.* **22**, 2000, 440–451.

\*KL Crenshaw, ME Peterson, Heeb, LA, et al.: Serum fructosamine concentration as an index of glycemia in cats with diabetes mellitus and stress hyperglycemia. *J Vet Intern Med.* **10**, 1996, 360–364.

\*R Duarte, Simoes, DMN, ML Franchini, et al.: Accuracy of serum beta-hydroxybutyrate measurements for the diagnosis of diabetic ketoacidosis in 116 dogs. *J Vet Intern Med.* **16**, 2002, 411–417.

\*DA Elliott, RW Nelson, CE Reusch, et al.: Comparison of serum fructosamine and blood glycosylated hemoglobin concentrations for assessment of glycemic control in cats with diabetes mellitus. *J Am Vet Med Assoc.* **214**, 1999, 1794–1798.

\*PA Graham, CT Mooney, M Murray: Serum fructosamine concentrations in hyperthyroid cats. *Res Vet Sci.* **67**, 1999, 171–175.

\*Hess RS, Kass PH, Ward CR: Breed distribution of dogs with diabetes mellitus admitted to a tertiary care facility. *J Am Vet Med Assoc* 200;216:1414-1417.

\*A Loste, MC Marc: Study of the effect of total serum protein and albumin concentrations on canine fructosamine concentration. *Can J Vet Res.* **63**, 1999, 138–141.

\*DK Macintire, Vincent-Johnson, NA, CW Kane, et al.: Treatment of dogs infected with *Hepatozoon americanum*: 53 cases (1989-1998). *J Am Vet Med Assoc.* **218**, 2001, 77–82.

\*MC Marca, A Loste, JJ Ramos: Effect of acute hyperglycaemia on the serum fructosamine and blood glycated haemoglobin concentrations in canine samples. *Vet Res Commun.* **24**, 2000, 11–16.

\*D Murphy, Reid, SWJ, PA Graham, et al.: Fructosamine measurement in ponies: Validation and response following experimental cyathostome infection. *Res Vet Sci.* **63**, 1997, 113–118.

\*R Nelson: Editorial: Stress hyperglycemia and diabetes mellitus in cats. *J Vet Intern Med.* **16**, 2002, 121–122.

\*JS Rand, E Kinnaird, A Baglioni, et al.: Acute stress hyperglycemia in cats is associated with struggling and increased concentrations of lactate and norepinephrine. *J Vet Intern Med.* **16**, 2002, 123–132.

\*SI Thoresen, WP Bredal: An evaluation of serum fructosamine as a marker of the duration of hypoproteinaemic conditions in dogs. *Vet Res Commun.* **22**, 1998, 167–177.

\*Valberg SJ, Ward TL, Rush B, et al: Gycogen branching enzyme deficiency in quarter horse foals. *J Vet Intern Med* 201; 15:572-580.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

## 13.6.5 Lipid

\*RF Butterwick, M McConnell, PJ Markwell, et al.: Influence of age and sex on plasma lipid and lipoprotein concentrations and associated enzyme activities in cats. *Am J Vet Res.* **62**, 2001, 331–336.

\*GK Ogilvie, RB Ford, DM Vail, et al.: Alterations in lipoprotein profiles in dogs with lymphoma. *J Vet Intern Med.* **8**, 1994, 62–66.

\* New references added to the third edition.

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## 14 Chapter 13 Evaluation of Renal Function and Urine

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*One must attend in medical practice not primarily to plausible theories but to experience combined with reason.*

Hippocrates of Cos, Greek physician, called *the Father of Medicine*, circa 460-377 BC

### 14.1 TESTS DEPENDENT ON RENAL FUNCTION

#### 14.1.1 Urea, Urea Nitrogen, and Creatinine (Figs. 13-1 to 13-6; see Fig. 10-8; Boxes 13-1 to 13-3; Case 13-1, see Cases 10-1, 10-2, 11-1, 12-1)

Urea is formed from ammonia by hepatic enzymes of the urea cycle during the process of protein metabolism. The urea enters the circulation, and most of it is freely filtered and excreted by the kidneys. Although it is neither actively reabsorbed nor secreted by the renal tubule, urea is highly diffuse. It passively moves into the interstitium of the kidney and returns to the circulation. The passive diffusion of urea is related to urine flow rate. An increase in flow rate (e.g., intravenous diuresis) results in a reduction of its level in the circulation. An increase in protein catabolism can increase the rate of urea formation. Causes of protein catabolism include high-protein diets, intestinal hemorrhage, and the prolonged administration of corticosteroids. Conversely, urea formation is reduced by a low-protein diet.

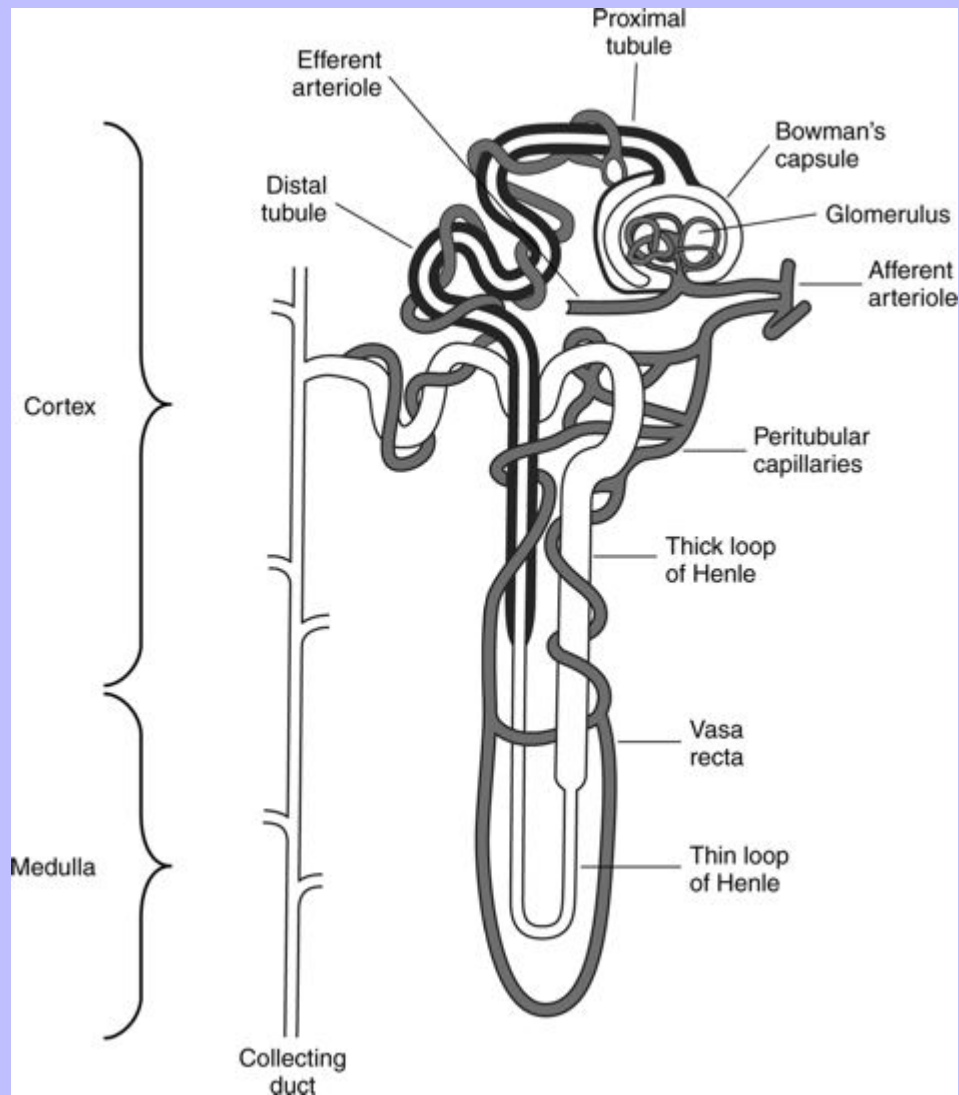
An increased level of urea in the circulation, termed *azotemia*, can be caused by prerenal, renal, or postrenal disorders. Prerenal azotemia is associated with conditions that reduce the glomerular filtration rate (GFR), thereby reducing the renal tubule flow rate. There is enhanced back-diffusion of urea into the peritubular interstitium with a subsequent increase in its level in the circulation. Renal azotemia is usually caused by severe kidney disease that adversely affects the number and/or microanatomy of the glomeruli, resulting in a reduction of the GFR. Postrenal azotemia is usually due to an obstructive process involving the urinary tract. The impaired ability to pass urine causes backpressure that increases back-diffusion of urea into the peritubular interstitium. The microflora of the rumen can metabolize urea that has back-diffused into the circulation and may attenuate the magnitude of its increase.

The majority of creatine is synthesized in the liver and transported to skeletal muscle where a portion is phosphorylated to form phosphocreatine. Creatine is the predominant storage compound of high-energy phosphate, and phosphocreatine serves as the high-energy source needed for muscle metabolism. Creatinine is an end product of creatine metabolism that is spontaneously formed by irreversible nonenzymatic dehydration of phosphocreatine. Creatinine diffuses into the circulation at a relatively constant rate, proportionate to muscle mass, and is freely filtered through the glomeruli. Although a portion of it is also secreted by renal tubules into the glomerular filtrate in humans, no appreciable secretion occurs in dogs or horses. Creatinine metabolism is generally spared from most extrarenal metabolic effects that can affect the level of urea in the circulation.

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Fig. 13-1



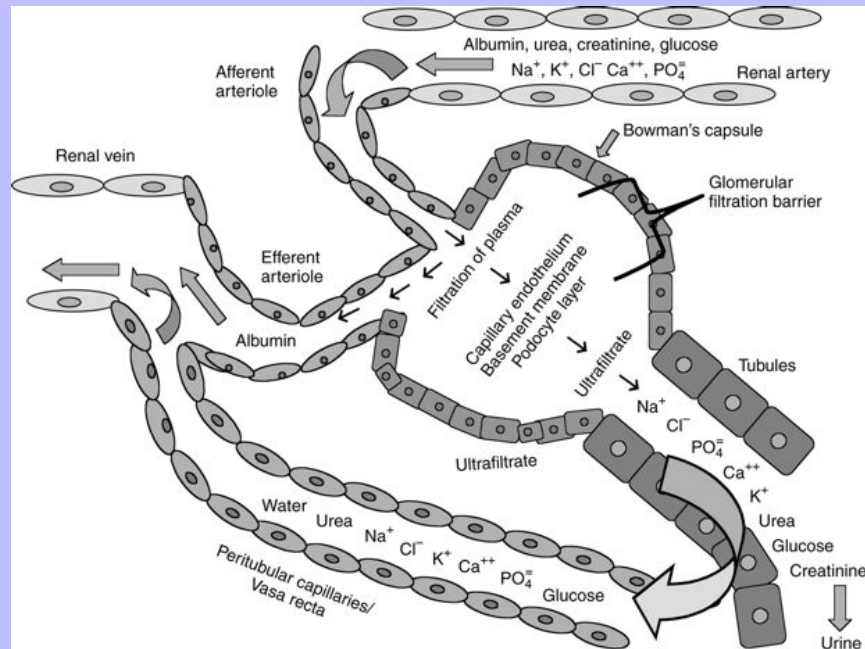
The renal artery eventually branches into the afferent arterioles, which form the functionally specialized tuft of capillary loops called *the glomerulus*. The capillaries coalesce to form the efferent arteriole. Its diameter is much smaller than that of the afferent arteriole, an arrangement that maintains pressure within the glomerulus in support of the filtration process. The glomerular perfusion pressure is relatively constant and is maintained independently of the systemic blood pressure. The efferent arteriole gives rise to peritubular capillaries and the vasa recta that pass around the tubules in the cortex and medulla. The vessels provide oxygen and nutrients to these structures and remove ions, molecules, and water after their reabsorption by the various segments of the renal tubules. The efferent arterioles progressively merge to form the renal veins, which drain into the inferior vena cava.

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The nephron is the functional unit of the kidney. It consists of a glomerulus (a tuft formed of capillary loops at the beginning of each tubule), proximal tubule, loop of Henle, distal tubule, and collecting duct. The collecting ducts progress to form the renal calyces where urine collects with subsequent passage into the urinary bladder via the ureters. The renal cortex is predominantly composed of glomeruli, proximal tubules, and distal tubules. The medulla contains the long radiating loops of Henle and collecting ducts with their associated vasa recta that point toward the renal pelvis. The proximal tubule reabsorbs the majority of filtered sodium, chloride, potassium, glucose, bicarbonate, phosphate, and sulfate and excretes most of the hydrogen. The main role of the loop of Henle is to provide the ability to generate concentrated urine. The distal tubular cells contain either sodium-potassium-adenosine triphosphatase activity or carbonic anhydrase activity. The distal tubule reabsorbs sodium chloride, secretes and reabsorbs potassium, and excretes hydrogen. The collecting ducts are formed from the distal tubules and ultimately drain into the renal calyx. The collecting ducts, normally impermeable to water, become permeable in the presence of antidiuretic hormone. Water is drawn into the medullary interstitium by the high osmotic pressure generated by the countercurrent multiplier system and returned to the circulation by the vasa recta. In summary, the tubular functions of reabsorption or secretion alter the glomerular filtrate, with urinary excretion as the end product.

Fig. 13-2



The glomerulus with its capsule (Bowman's) constitutes the corpusculum renis (malpighian body). The endothelial cells of the capillaries, inner lining of epithelial cells (podocytes) of Bowman's capsule, and basement membrane form the glomerular filtration barrier. The endothelial cells are perforated by numerous, relatively large fenestrations (pores), which are lined with a surface coating of fixed negatively charged glycoprotein (podocalyxin) that impairs the passage of plasma proteins. The basement membrane consists of three layers. One layer consists of a felt-like composition of type IV collagen fibrils embedded in a gel-like matrix of glycoprotein (fibronectin, laminin) and proteoglycans that forms the main size-dependent barrier to passage of protein into the ultrafiltrate. The other two layers are rich in heparan sulfate, a negatively charged polyanionic glycoprotein, that forms the main charge-dependent barrier to the passage of plasma protein into the ultrafiltrate. Podocytes are the inner (visceral) layer of epithelial cells of Bowman's capsule that eventually become continuous with it. Bowman's capsule progresses into the formation of the proximal tubule. Podocytes embrace the outer surface of the tufts of capillaries. Podocytes are not contiguous but have numerous interdigitating cytoplasmic extensions embedded in the basement membrane that form filtration slits (pores) through which the filtrate moves. An anionic mucopolysaccharide gel that is rich in sialic acid coats the slits, forming a physiochemical barrier that is relatively impermeable to most proteins with molecular weights greater than approximately 60,000 d. Podocytes contain actin filaments, lysosomes, and microtubules. In addition to elaborating constituents of the basement membrane, they may have contractile and phagocytic functions.

Within the glomerulus are mesangial cells that have phagocytic and contractile functions. They remove circulating immune complexes and pose a risk for the development of glomerular pathology. The myofilaments of the cell are stimulated to contract by angiotension II and arginine vasopressin, which reduces the available filtration surface, resulting in an alteration of the glomerular filtration rate.

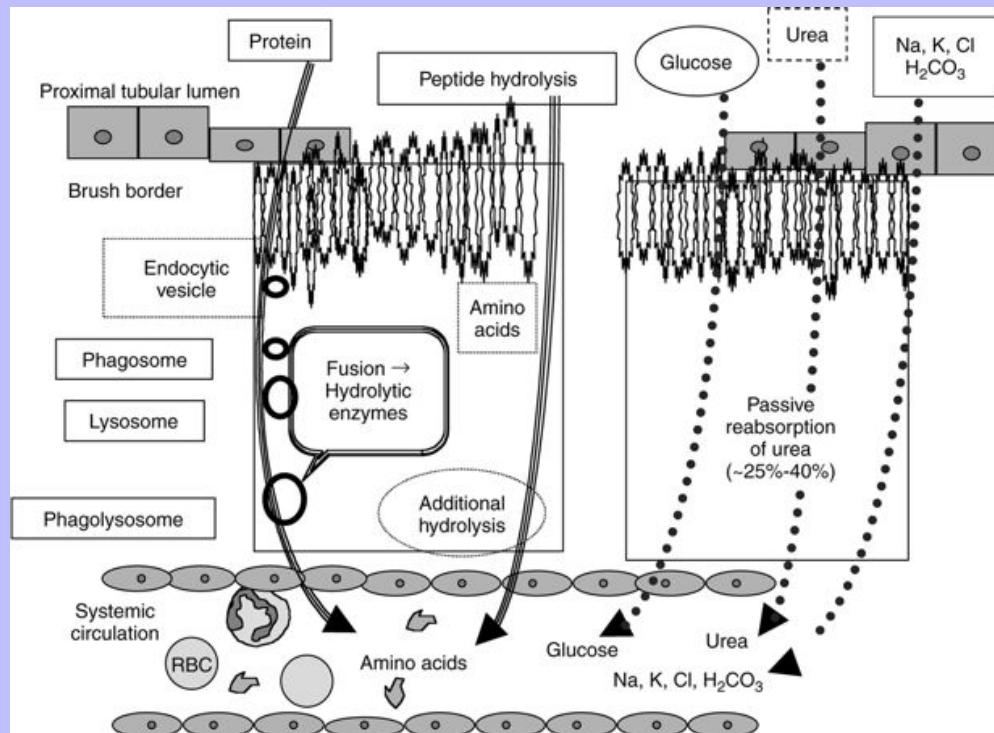
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An increased level of creatinine in the circulation is generally due to disorders that cause a reduction of the GFR (prerenal), severe kidney disease that adversely affects the number and/or microanatomy of the glomeruli (renal), and obstructive disorders that impair its elimination in urine. Since ruminant microflora do not metabolize creatinine, its increase in the circulation is not attenuated in association with creatinine-raising disorders.

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Fig. 13-3



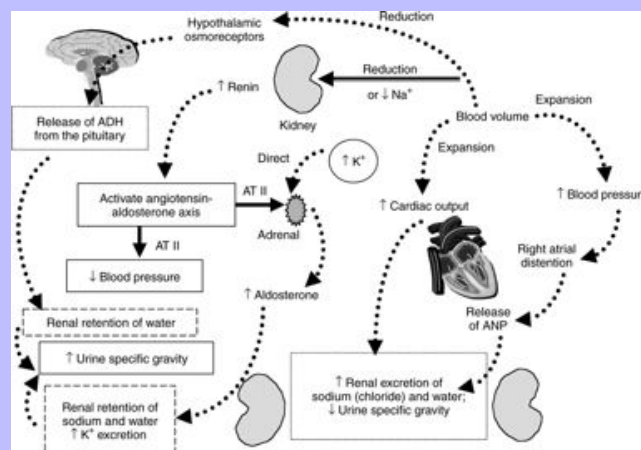
The renal tubule reabsorbs the filtered smaller proteins (insulin, growth hormone), glucose, and the electrolytes. The reabsorbed proteins are metabolized by a catabolic process that involves incorporation into endocytic vesicles (endosomes), their fusion with lysosomes, and lysosomal-enzyme hydrolysis of the proteins to amino acids with their return to the systemic circulation. Small peptides (8 to 10 amino acids) in the ultrafiltrate can be hydrolyzed at the luminal surface of the brush border. The reabsorption of abnormally large quantities of peptides or small proteins can cause tubular damage. Multiple myeloma is one example in which the large quantity of filtered light chain immunoglobulins can precipitate (perhaps in combination with Tamm-Horsfall protein) and cause tubular obstruction and damage. The condition is referred to as *light chain cast nephropathy*.

Comment: The urea level in serum or plasma is commonly determined by either enzymatic methods that are based on the hydrolysis of urea with urease or chemical methods that are based on the formation of a chromogen. Since whole blood is not used in these assays, the term *blood urea nitrogen (BUN)* is technically incorrect. However, the acronym is entrenched in laboratory medicine.

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The kidneys are involved in homeostasis of calcium, phosphate, potassium, and sodium and the acid-base balance. Comments pertaining to a change in their levels in the circulation are generally restricted to those that are a consequence of renal insufficiency. Hypocalcemia can develop in chronic renal disease. Hypercalcemia frequently occurs in horses and occasionally in dogs with advanced renal disease. Hypercalcemia can also be a cause of kidney disease. Acute or chronic disorders that result in a reduction in the GFR can cause hyperphosphatemia. Hyperkalemia is associated with postrenal obstructive disorders and oliguria. Hypokalemia commonly develops in cats with chronic renal disease. Hyponatremia can develop in chronic renal failure ("salt-losing nephropathy"). Metabolic acidosis is a common complication of renal failure. Hyperlipidemia develops in association with the nephrotic syndrome, proteinuria, hypoalbuminemia, hypercholesterolemia, and edema. Hypercholesterolemia is likely due to renal loss of lipoproteins that carry cholesterol from tissues to the liver for elimination or a compensatory increase in hepatic synthesis of lipoproteins along with other proteins in response to those lost in the urine. 228 229

Fig. 13-4



The kidneys have a key role in the regulation of the circulating fluid volume and the blood pressure. The cells of the loop of Henle and the afferent arteriole show regional specialization where the ascending tubule passes close to the glomerulus. The tubule forms the macula densa, and the arteriolar cells are filled with granules containing renin and innervated with sympathetic nerve fibers; the area is called *the juxtaglomerular apparatus*. The juxtaglomerular apparatus is the predominant regulator of systemic blood pressure through modulation of the circulating intravascular blood volume and sodium concentration. Renin is released in response to carotid artery barometers as a result of detection of a reduction in circulating volume in combination with decreased afferent arteriolar pressure and reduced intraluminal sodium delivery to the macula densa. Renin release from the macula densa is mediated by renal cortical prostaglandins, predominantly prostaglandin I<sub>2</sub>. Renin, a proteolytic enzyme, converts the circulating protein, angiotensinogen, into angiotensin I with subsequent formation of angiotensin II in the lungs. Angiotensin II is a potent vasoconstrictor, and the increased activity of the renin-angiotensin system increases the secretion of aldosterone by the adrenal glands. The vasoconstriction signals the hypothalamic osmoreceptors to enhance the release of antidiuretic hormone (ADH, arginine vasopressin). The antidiuretic hormone-mediated water retention and the aldosterone-mediated increased renal tubular reabsorption of sodium result in increases in the intravascular volume and blood pressure. Angiotensin II has an inhibitory effect on renin release as part of a negative feedback loop.

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*One can obtain considerable information concerning the general trends by examining urine.*

Hippocrates of Cos, Greek physician, called *the Father of Medicine*, circa 460-377 BC

The functional unit of the kidney is the nephron, a long convoluted tubular structure consisting of the glomerulus, the proximal convoluted tubule, the nephronic loop, and the distal convoluted tubule. The glomerulus is composed of a tuft of capillary loops at the beginning of each nephric tubule and is surrounded by Bowman's capsule. It forms the glomerular filtrate that is subsequently altered by the tubule, resulting in the formation of urine.

## 14.2 URINALYSIS

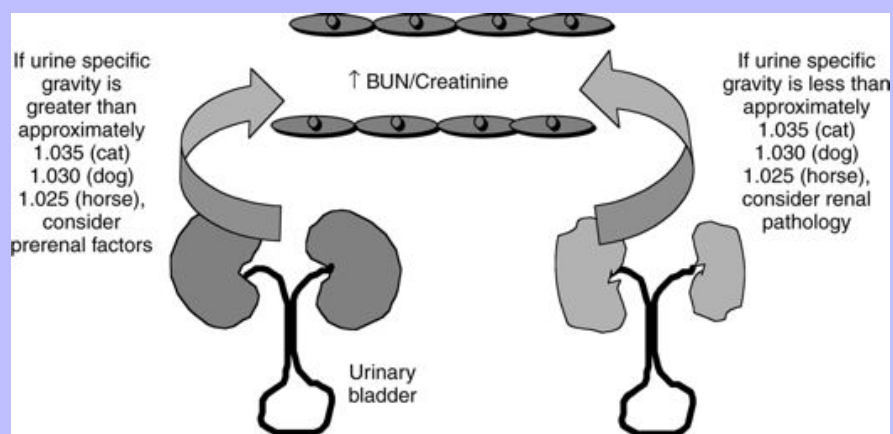
### 14.2.1 Appearance and Specific Gravity

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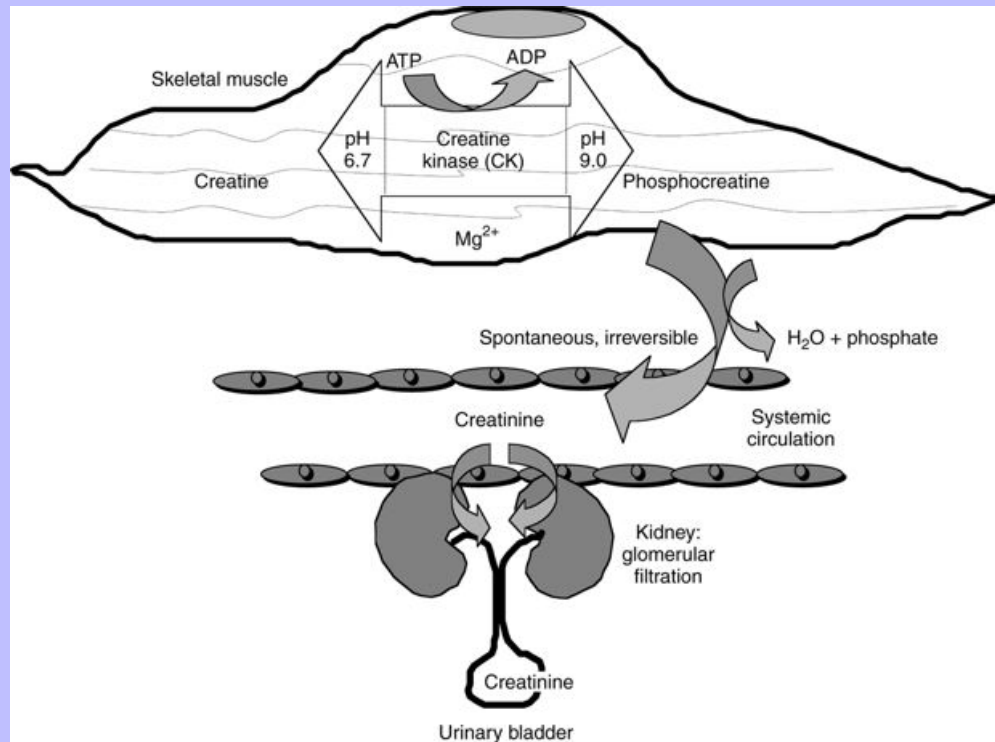
Urine can be collected by cystocentesis, catheterization, or free catch (voided). Samples collected by cystocentesis are preferred because contamination from the genitourinary tract is avoided. The most accurate results are obtained by testing freshly collected urine or samples that have been refrigerated for a short time. Dog and cat urine generally has a faint aroma. Bacterial infections impart an ammonia-like, fetid odor. Antibiotics and food supplements can impart odor to urine. The normal yellow coloration of urine is due to urobilin (urochromes). Exposure of urine with increased levels of colorless urobilinogen to light results in the formation of increased levels of urobilin, which imparts a dark yellow to orange color. A yellow-brown or green-brown color is generally due to bilirubin. The presence of blood, hemoglobin, or myoglobin imparts a red to red-brown color. Dark brown urine may be due to the formation of methemoglobin from hemoglobin during prolonged standing. Urine is generally transparent. Cloudy urine may be due to the presence of cells, bacteria, fat, crystals, or mucus. The cause of cloudy urine can be determined by microscopic examination of sediment. Horse urine normally contains mucus and calcium carbonate crystals that impart viscous, opaque properties.

Fig. 13-5



A general relationship of the urine specific gravity and the circulating blood urea nitrogen (*BUN*) and creatinine levels for the tentative differentiation of prerenal azotemia and renal disease is illustrated.

Fig. 13-6



Skeletal muscle contains high activities of creatine kinase (*CK*) in the cytoplasm. Creatine kinase catalyzes the pH-dependent reversible phosphorylation of creatine by adenosine triphosphate (*ATP*) to form phosphocreatine (creatine phosphate), the high-energy source for muscle contraction. Magnesium ( $Mg^{2+}$ ) is a necessary activating ion that functions within a narrow concentration range. Creatinine is an end product of creatine metabolism that is spontaneously formed by irreversible nonenzymatic dehydration of phosphocreatine. Creatinine diffuses into the circulation at a relatively constant rate, proportionate to muscle mass, and is freely filtered through the glomeruli. *ADH*, Adenosine diphosphate.

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## 14.2.1.1 BOX 13-1 Markers of Renal Tubular Injury

<b>Casts</b>		
<b>Type of Cast</b>	<b>Possible Cause/Association</b>	<b>Interpretation</b>
Hyaline	Proteinuria (renal or extrarenal)	Mild to severe disease
Epithelial	Ischemia, nephrotoxins	Acute, severe injury
Granular, fatty, waxy	Ischemia, nephrotoxins	Combination of Tamm-Horsfall protein plus degenerated epithelial cells or leukocytes (chronic)
Leukocyte	Inflammation	Infection involving tubules
Erythrocyte	Hemorrhage	Bleeding in the kidney
Broad	Tubular obstruction/dilatation	Renal disease (failure)
<b>Enzyme/Creatinine Ratios *</b>		
<b>Species GGT/creatinine NAG/creatinine</b>		
Dog	0.39 ± 0.18	0.06 ± 0.04
Horse	10.52 ± 4.78	
Sheep	0.015 ± 0.008	$1.5 \times 10^{-3} \pm 6 \times 10^{-4}$
GGT, $\gamma$ -Glutamyl transferase; NAG, N-acetylglucosaminidase.		

\* Enzyme (U/L) divided by creatinine (mg/dL). Expressed as mean ± SD.

## 14.2.1.2 BOX 13-2 Causes of Azotemia (Increased Urea Nitrogen and/or Creatinine)

### 14.2.1.2.1 Prerenal

Dehydration

Cardiovascular disease

Shock (septic or traumatic)

High-protein diet (urea-nitrogen increase only)

Hemorrhage into gastrointestinal tract (urea-nitrogen increase only)

### 14.2.1.2.2 Renal

Renal diseases causing two thirds to three fourths of nephrons to be destroyed

### 14.2.1.2.3 Postrenal

Obstruction of urinary tract

Rupture of urinary tract

Specific gravity indicates the density of urine, the relative proportion of dissolved solid constituents to total volume of the specimen. Large particles such as protein and glucose have a greater effect on the value measured than smaller components such as electrolytes. In contrast, osmolality indicates the number of solute particles per unit of solution. Its measurement is preferred in certain metabolic disorders. In most situations, the

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specific gravity has a constant relationship to osmolality. The specific gravity is a useful indicator of the ability of the renal mechanisms to concentrate urine. The specific gravity of the glomerular filtrate is approximately 1.008 to 1.012. A urine specific gravity greater than 1.012 or less than 1.008 requires tubular cell function and generally reflects the hydration status of the animal. Urine volume in dogs and cats ranges from 20 to 40 and 20 to 30 mL/kg body weight every 24 hours, respectively. Concomitant evaluation of the urine specific gravity and the blood urea nitrogen level and/or creatinine level in the circulation provides important interdependent information about kidney function.

## 14.2.1.3 BOX 13-3 Electrolyte and Nonelectrolyte Changes in Advanced Renal Disease

Renal Alteration	Result	
<b>Electrolytes</b>		
Sodium	↑ Fractional excretion	Hyponatremia
Potassium	↓ Fractional excretion	Hyperkalemia
Bicarbonate	↓ Conservation	Acidemia
Phosphate	↓ Excretion	Hyperphosphatemia (dog and cat)
Calcium	↑ Excretion as phosphate ↑	Hypocalcemia
<b>Nonelectrolytes</b>		
Blood pH	↓ Removal of H <sup>+</sup> and acid products, bicarbonate loss	Acidemia
Serum proteins	Persistent proteinuria	Hypoalbuminemia

## 14.3 REAGENT STRIP TESTING

### 14.3.1 pH

The kidneys and lungs maintain acid-base homeostasis. Renal tubules reabsorb and generate bicarbonate, secrete ammonium ions, and exchange hydrogen ions for sodium. Urine contains acids such as phosphoric and sulfuric acids complexed as salts from systemic metabolism. Urine pH is measured as a diagnostic adjunct and as a means of monitoring treatment. The reagent strip (dipstick) method, pH paper, and pH meters are used to measure urine pH. The latter generally provides the most reliable measurement. Portable pH meters are practical instruments that provide accurate values for feline and food animal urine and food animal rumen fluid. Adherence to electrode maintenance and calibration instructions is essential. Feline urine can be refrigerated for up to 24 hours before testing without adverse effect.

Urine pH is influenced by diet. Herbivorous animals have an alkaline pH, and the urine pH in carnivores and omnivores will vary from acid to alkaline, depending on the amount of animal protein in the diet. Acid urine may be caused by metabolic or respiratory acidosis, starvation, fever, prolonged muscular exercise, or the administration of acid salts such as ammonium chloride. Causes of alkaline urine include metabolic or respiratory alkalosis, bacterial cystitis, and administration of sodium bicarbonate.

### 14.3.2 Protein

Detection of an abnormal quantity of protein in the urine is an indicator of kidney disease. Microalbuminuria is the presence of an abnormal level of albumin in urine that is not detected by conventional reagent strip (dipstick) methods. Detection of microalbuminuria is an early and sensitive indicator of many types of chronic kidney disease (glomerular pathology) in humans. Conventional reagent strip methods generally detect urine protein concentrations that are greater than 30 mg/dL (300 µg/mL). A canine albumin-capture enzyme-linked immunosorbent assay (ELISA) method indicates that microalbuminuria in the dog is defined as 1 to 30 mg/dL

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(10 to 300 µg/mL). Immunologic test systems (e.g., Micral II test strip; Boehringer Mannheim, Indianapolis, IN) and dye-binding chemical test strips (e.g., Clinitek microalbumin; Bayer Diagnostics, Tarrytown, NY) that are used in human laboratory medicine do not appear to be reliable screening tests for the detection of canine microalbuminuria.

The urine specific gravity will affect the reagent strip value through either concentration or dilution effects.

Increased values can be further explored by determining the urine protein to urine creatinine ratio. Values 232

greater than 1 are generally considered abnormal in dogs and cats. The presence of blood or inflammation will produce a positive result because of the contribution of albumin from the circulation. 233

Bence Jones proteinuria is most often associated with multiple myeloma. These immunoglobulin light chains easily slip through the glomeruli. Because of the specificity of the reagent strip pad method for measurement of albumin, Bence Jones proteins are not detected. Determination of the urine protein by the sulfosalicylic acid method (Bumintest [Ames Company, Elkhart, Ind.]) will usually detect Bence Jones protein, as well as albumin.

Small proteins such as  $\alpha_1$ -microglobulin (30,000  $M_r$ ),  $\beta_2$ -microglobulin (12,000  $M_r$ ), lysozyme (14,000  $M_r$ ), and retinol-binding protein (21,000  $M_r$ ) are normally present in the glomerular filtrate, and reabsorbed by the renal tubules and catabolized. When the renal tubules are injured, the proteins can be detected in the urine by protein electrophoresis. Their presence is not detected by either the reagent strip test or sulfosalicylic acid method. Tubular proteinuria is an early indicator of damage to that segment of the nephron without concomitant change in the blood urea nitrogen level and/or creatinine level in the circulation. Some causes include drugs (aminoglycosides, cyclosporine), heavy metals (lead), analgesics (nonsteroidal antiinflammatory drugs), ischemia, and metabolic diseases (Fanconi-like syndrome).

### 14.3.3 Glucose (see [Fig. 12-13](#))

Glucose is freely filtered and is completely reabsorbed by the tubules. Glycosuria or glucosuria refers to the presence of glucose in urine. The glucose oxidase-impregnated reagent strip pad detects glycosuria when the filtered glucose load exceeds the tubular reabsorption mechanism or when tubular dysfunction or injury is present. False-negative reactions may occur in the presence of ascorbic acid. Dogs can synthesize variable amounts of ascorbic acid. The commercial tablet (Clinitest; Ames Company) that detects reducing substances is not affected by ascorbic acid. Cystitis in cats may also produce a false-positive reaction with the reagent strip method.

When the glucose level in the circulation is not increased, renal glycosuria indicates tubular dysfunction. Primary renal glycosuria has been reported as an isolated finding in Scottish terriers, Norwegian elkhounds, and mixed breeds with detectable underlying disease. Renal glycosuria may be a component of more general renal tubular dysfunction referred to as *the Fanconi-like syndrome*. The disorder may be inherited or acquired. Other constituents that may be concomitantly lost in the urine include amino acids, phosphate, and bicarbonate. Tubular dysfunction has been reported in basenji greyhounds, Norwegian elkhounds, Shetland sheepdogs, and miniature schnauzers and in association with certain nephrotoxic drugs.

### 14.3.4 Ketones (see [Fig. 12-13](#))

Excessive ketone formation results from accelerated oxidation of fatty acids as an energy source. The prolonged absence of circulating insulin in diabetes and actions of the counter-regulatory hormones actuate the formation ketone bodies, acetoacetate and  $\beta$ -hydroxybutyrate, with subsequent appearance in urine referred to as *ketonuria*. Although increased levels of both ketone bodies in the circulation indicate the presence of ketosis, an

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increase in the latter is a more accurate indication of ketoacidosis. The urine reagent strip test (nitroprusside reaction) detects acetoacetate but does not react with  $\beta$ -hydroxybutyrate. The measurement of the  $\beta$ -hydroxybutyrate level in the circulation is an accurate reflection of the presence ketoacidosis in diabetic dogs. Two relatively common disorders resulting in ketonuria in ruminants are bovine ketosis and ovine pregnancy toxemia. Bovine ketosis occurs in high-producing dairy cows, probably as a consequence of inadequate hepatic production of glucose to meet energy needs. Pregnant ewes carrying twins and subjected to inadequate nutrition or stress become hypoglycemic at the expense of the fetuses and supplement their energy needs by increasing fat metabolism.

### 14.3.5 Occult Blood

A positive reaction to an occult blood test occurs with hematuria (erythrocytes in the urine), hemoglobinuria, and myoglobinuria. Hematuria indicates inflammation or hemorrhage in the urinary tract. The absence of red blood cells (RBCs) in the urine sediment and a second positive test result in the supernatant are generally indicative of hemoglobinuria or myoglobinuria. Chemical differentiation of hemoglobinuria and myoglobinuria is problematic. Hemoglobinuria is generally a consequence of hemolytic anemia; there is a concomitant reduction in the hematocrit. If the creatine kinase level in the circulation is increased, it is likely that muscle injury resulted in myoglobinuria.

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### 14.3.6 Bilirubin and Urobilinogen (see [Figs. 10-10, 10-11](#))

Conjugated bilirubin appears in urine if there is an increased level of the form that is noncovalently bound to albumin in the circulation. A trace or 1+ reaction in concentrated canine urine may be a normal finding. Urobilinogen lacks diagnostic utility.

### 14.3.7 Urine Enzymes

$\gamma$ -Glutamyltransferase and N-acetylglucosaminidase are two enzymes that have high activity in renal tubules. The measurement of their 24-hour excretion is a relatively sensitive and early marker of tubular injury in dogs, horses, and sheep. An enzyme to creatinine ratio determined from a single urine sample enhances its clinical application (see [Table 11-1](#)). Renal tubular damage (e.g., aminoglycoside toxicity) is associated with an increase in the enzyme to creatinine ratio before a change in the serum creatinine concentration, endogenous creatinine clearance, urine protein to creatinine ratio, and change in the urine specific gravity. The enzyme to creatinine ratio is affected by a reduction in GFR and inflammation in the urinary tract.

### 14.4 MICROSCOPIC SEDIMENT

A detailed description of findings in the urine sediment that is amply supported by photomicrographs is covered in other sources (see Additional Reading). Therefore only general comments pertaining to the evaluation of urine sediment are provided. Urine collected by cystocentesis is sterile and normally contains sparse numbers of cells and other formed elements from the urinary tract. The same volume of urine (e.g., 5 mL) is consistently used, and the same centrifugation time is used to provide a uniform foundation for the semiquantitative assessments. Sediment can be unstained or stained for microscopic examination. An unstained specimen reduces the likelihood of introducing bacteria and/or yeast contaminants that may be in stain. The sediment is reconstituted with either an equal volume of urine or stain, a small drop is placed on a clean glass slide, and a cover slip is applied. The condenser of the microscope is lowered to accentuate the microscopic recognition of the constituents in the sediment. Yeasts and fungal hyphae are usually contaminants introduced during the collection procedure or found

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in the stain. Sperm are found in some urine samples taken by cystocentesis from intact males and recently bred females.

## 14.4.1 Cells

Zero to three leukocytes per high-power field (hpf) (40× objective) and 0 to 3 erythrocytes/hpf are normal findings in urine collected by cystocentesis. Increased numbers of leukocytes (pyuria) or RBCs (hematuria) indicate inflammation in the urinary tract. Pyuria is often due to bacterial infection, and a specimen is submitted for culture. Leukocytes or RBCs entrapped in a cast indicate a renal origin. Uroliths are a common cause of blood in the urine. Leukocytes and/or erythrocytes in urine collected midstream may reflect hemorrhage or inflammation in the genital tract. Their presence in a second specimen collected by cystocentesis confirms a urinary source.

The presence of a small number of epithelial cells in urine is a normal finding. Increased numbers can be associated with inflammation or neoplasia of the urinary tract. Clumps of epithelial cells, especially if composed of cells showing moderate to marked variation in cell size or structure, require additional evaluation to determine whether they are cancerous. Another drop of reconstituted sediment is placed on a clean glass slide, air-dried, and stained for cytologic examination.

## 14.4.2 Casts

Casts represent an elongated or cylindrical mold formed in renal tubules by congealed protein and disintegrated epithelial cells. They rapidly disintegrate in urine left standing before examination. Hyaline, granular (fine and coarse), and waxy casts represent a continuum in cast formation and are the ones most commonly observed. A cellular cast is formed by the entrapment of erythrocytes, leukocytes, or exfoliated tubular/epithelial cells within the cast. The protein matrix is largely formed by Tamm-Horsfall protein, named for the two individuals who initially identified it in human urine. It is a membrane-associated renal glycoprotein that is predominantly produced in the thick ascending limb of the loop of Henle.

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Hyaline casts are relatively transparent and are the earliest cast form associated with kidney disease. Granular casts are relatively dark and are composed of increased amounts of particulate cellular debris within the protein matrix. Waxy casts consist of homogeneous proteinaceous material that has a high refractive index, in contrast to the low refractive index of hyaline casts. Their presence generally indicates an advanced stage of the disintegrative process of cast formation associated with kidney disease. The presence of occasional hyaline and granular casts per low-power field (10× objective) is a normal finding. Cylindruria refers to increased numbers of any type of cast. Fever and exercise can cause a small increase in hyaline casts. Increased numbers of hyaline, granular, and waxy casts are often observed in association with a variety of kidney diseases and insults. The presence of leukocytes, epithelial cells, or both within a cast is suggestive of pyelonephritis and/or acute tubular necrosis.

## 14.4.3 Crystals (see Fig. 10-9)

Refrigeration of urine can accentuate the formation of crystals. Examination of urine within 1 hour of collection reduces the storage-related effect of temperature and time on crystal formation. Certain crystals can be normal findings in dogs, cats, and horses. Struvite crystalluria in dogs and cats and calcium carbonate crystalluria in horses are common findings. Crystalluria may indicate urolithiasis. Certain crystals may be the initial indication of a systemic disorder. Ammonium urate crystalluria is associated with hepatic insufficiency, especially congenital portosystemic shunts. However, ammonium urate crystals are common in Dalmatians without liver

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insufficiency. Calcium oxalate monohydrate (coffin-like appearance with its lid angled partially open on one end; hippuric acid crystal– appearing form) crystalluria is associated with ethylene glycol (antifreeze) toxicosis.

## 14.5 ADDITIONAL READING\*

LG Adams, DJ Polzin, CA Osborne, et al.: Correlation of urine protein/creatinine ratio and twenty-four-hour urinary protein excretion in normal cats and cats with surgically induced chronic renal failure. *J Vet Intern Med.* **6**, 1992, 36–40.

R Adams, JJ McClure, KA Gossett, et al.: Evaluation of a technique for measurement of gamma glutamyltranspeptidase in equine urine. *Am J Vet Res.* **46**, 1985, 147–150.

\*H Albasan, JP Lulich, CA Osborne, et al.: Effects of storage time and temperature on pH, specific gravity, and crystal formation in urine samples from dogs and cats. *J Am Vet Med Assoc.* **222**, 2003, 176–179.

A Bernard, RR Lauwerys: Proteinuria: Changes and mechanisms in toxic nephropathies. *Crit Rev Toxicol.* **21**, 1991, 373–405.

\*M Cohen, GS Post: Water transport in the kidney and nephrogenic diabetes insipidus. *J Vet Intern Med.* **16**, 2002, 510–517.

AK Cook, LD Cowgill: Clinical and pathological features of protein-losing glomerular disease in the dog: A review of 137 cases (1985–1992). *J Am Anim Hosp Assoc.* **32**, 1996, 313–322.

F Garry, DJ Chew, GF Hoffis: Enzymuria as an index of renal damage in sheep with induced aminoglycoside nephrotoxicosis. *Am J Vet Res.* **51**, 1990, 428–432.

GF Grauer, DS Greco, EN Behrend, et al.: Estimation of quantitative enzymuria in dogs with gentamicin-induced nephrotoxicosis using urine enzyme/creatinine ratios from spot urine samples. *J Vet Intern Med.* **9**, 1995, 324–327.

R Heiene, WJ Biewenga, JP Koeman: Urinary alkaline phosphatase and gamma glutamyltransferase as indicators of acute renal damage in dogs. *J Small Anim Pract.* **32**, 1991, 521–524.

JR Hoyer, MW Seiler: Pathophysiology of Tamm-Horsfall protein. *Kidney Int.* **16**, 1979, 279–289.

\*WF Kean, G Eknoyan: Proteinuria, albuminuria, risk assessment, detection, elimination (PARADE): A position paper of the National Kidney Foundation. *Am J Kidney Dis.* **33**, 1999, 1004–1010.

JP Lulich, CA Osborne: Interpretation of urine protein-creatinine ratios in dogs with glomerular and nonglomerular disorders. *Comp Contin Educ Small Anim Pract.* **12**, 1990, 59–71.

DJ Meyer: Microscopic examination of the urinary sediment. In Raskin, RE, Meyer, DJ (Eds.): *Atlas of canine and feline cytology*. 2001, WB Saunders Co, Philadelphia, 261–276.

\*G Nappert, JM Naylor: A comparison of pH determination methods in food animal practice. *Can Vet J.* **42**, 2001, 364–367.

Norden, AGW, LM Fulcher, M Lapsley, et al.: Excretion of beta 2-glycoprotein I (apolipoprotein H) in renal tubular disease. *Clin Chem.* **37**, 1991, 74–77.

TM Ortega, EC Feldman, RW Nelson, et al.: Systemic arterial blood pressure and urine protein/creatinine ratio in dogs with hyperadrenocorticism. *J Am Vet Med Assoc.* **209**, 1996, 1724–1729.

\*BM Pressler, SL Vaden, WA Jensen, et al.: Detection of canine microalbuminuria using semiquantitative test strips designed for use with human urine. *Vet Clin Pathol.* **31**, 2002, 56–60.

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# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

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\*RE Raskin, KA Murray, JK Levy: Comparison of home monitoring methods for feline urine pH measurement. *Vet Clin Pathol.* **31**, 2002, 51–55.

BJ Rivers, PA Walter, TD O'Brien, et al.: Evaluation of urine gamma-glutamyltranspeptidase-to-creatinine ratio as a diagnostic tool in an experimental model of aminoglycoside-induced acute renal failure in the dog. *J Am Anim Hosp Assoc.* **32**, 1996, 323–336.

AE Schultze, RD Jensen: Sodium dodecyl sulfate polyacrylamide gel electrophoresis of canine urinary proteins for the analysis and differentiation of tubular and glomerular diseases. *Vet Clin Pathol.* **18**, 1990, 93–97.

KV Waller, KM Ward, JD Mahan, et al.: Current concepts in proteinuria. *Clin Chem.* **35**, 1989, 755–765.

\*JH Warren, G Gearin, L Laffel, et al.: Effect of duration of type I diabetes on the prevalence of stages of diabetic nephropathy defined by urinary albumin/creatinine ratio. *J Am Soc Nephrol.* **7**, 1996, 930–937.

MH Weber: Proteinuria: Causes, forms and methods of determination. *Clin Diagn Lab Med.* **1**, 1988, 49–55.

\* New references added to the third edition.

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## 15 Chapter 14 Evaluation of Electrolyte and Acid-Base Disorders

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*When we try to pick out something by itself, we find it hitched to everything else in the universe.*

**John Muir, 1838-1914, British-born American naturalist who promoted the creation of national parks**

### 15.1 BODY FLUID COMPARTMENTS (Fig. 14-1, see Figs. 13-2, 13-4, Fig. 15-1)

Approximately 60% of an adult's body weight is water; 40% is intracellular and 20% is extracellular. Of the extracellular water, 5% is in the plasma compartment and 15% is in the interstitium. Sodium is the principal cation in the extracellular fluid, and potassium is the principal intracellular cation. More than 98% of the total body potassium is located within cells. The principal intracellular anion is phosphate. Chloride and bicarbonate are the predominant anions in the extracellular space. The water content of plasma and electrolyte homeostasis are primarily coordinated by integrated functions involving endocrine organs, the nervous system, and the kidneys. The renal tubules are generally responsible for executing the neurohormonal instructions by preserving sodium and water and eliminating potassium. Proximal tubules and distal tubules execute sodium reabsorption. The distal tubular exchange of sodium and potassium is accelerated by aldosterone, promoting sodium retention and potassium excretion.

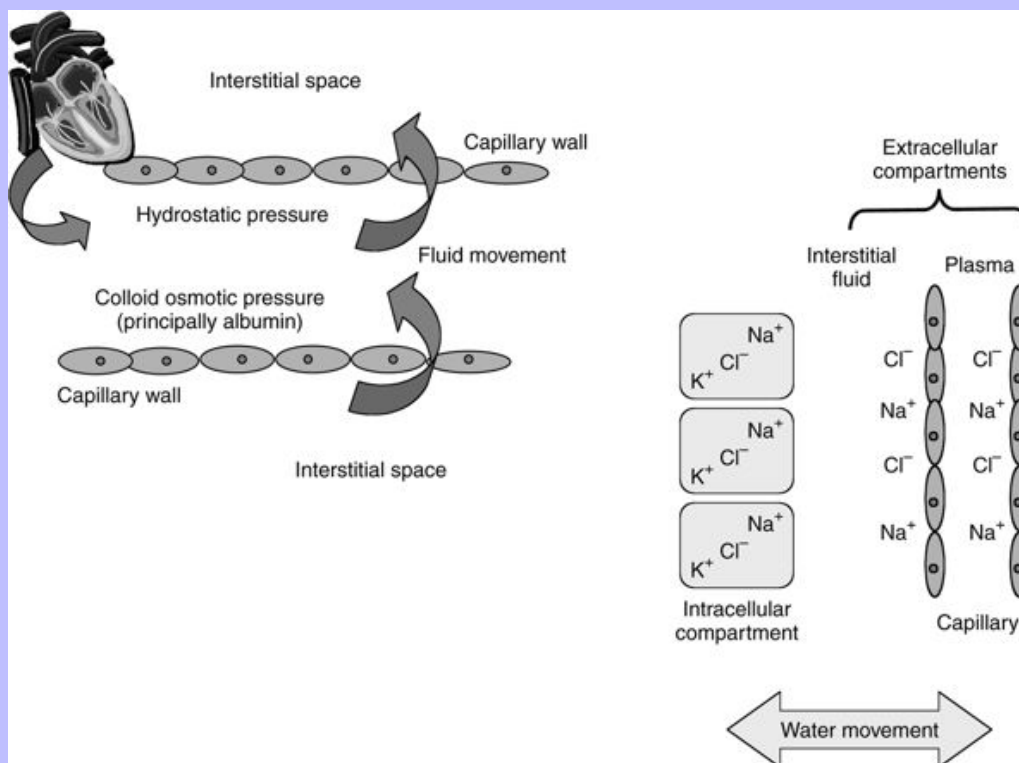
The amount of pressure required to counterbalance the osmotic movement of water across a selectively permeable membrane is called *osmotic pressure*. Its measurement is an indirect reflection of the water and solute concentrations of a solution. The osmotic pressure is directly proportional to the concentration of osmotically active particles in solution. One molecule of sodium chloride has twice the osmotic effect of one molecule of albumin. An increased osmotic pressure indicates a higher solute concentration and a lower water concentration. The osmolal concentration of solution is called *osmolality* and is expressed as osmoles per kilogram of water. For measurements in biologic fluids such as plasma and urine, the term *milliosmole* (mOsm), 1000 osmole, is conventionally used. Osmolarity, osmoles per liter of solution, effectively equates to osmolality in biologic fluids, and the terms can be used interchangeably. The measurement of osmolarity is used to determine whether there are clinically important changes in the total body water balance. The osmotic pressure of a solution is proportional to its osmolality because of its solute concentration. Sodium and chloride constitute more than 90% of the solute in the extracellular fluid. The circulating sodium concentration is generally an indicator of plasma osmolality. Its

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measurement in concert with two prominent nonelectrolyte constituents in the circulation that influence osmolality, glucose and urea, can be used to calculate osmolarity. Calculated osmolarity (mOsm/kg) is two times sodium (mmol/L) plus glucose (mmol/L) plus urea (mmol/dL). Sodium concentration (in millimoles per liter) is determined by dividing the sodium value (expressed as mEq/L) by 1, the glucose value (mg/dL) by 18, and the urea value (mg/dL) by 2.8. Osmolarity is measured by special instrumentation. The difference between the measured osmolality and calculated osmolarity represents the small amount of circulating constituents that are detected by the direct measurement but not included in the calculation. This difference, or osmolal gap, is relatively consistent. A difference of greater than 10 mOsm/kg suggests the presence of foreign substances, notably ethylene glycol, ethanol, and methanol.

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Fig. 14-1



The balance of hydrostatic and colloid osmotic pressure across the capillary wall primarily maintains the normal extracellular fluid distribution between plasma and interstitial spaces. The concentration of albumin in the circulation is the predominant factor for maintenance of colloid osmotic pressure because of its small size and abundance. Hepatic synthesis of albumin is primarily controlled by colloidal osmotic pressure when nutrition is adequate. Altered hydrostatic pressure, severe hypoalbuminemia, or altered integrity of the capillary membrane may result in edema or effusion formation.

The distribution of fluid between intracellular and extracellular compartments is primarily determined by the osmotic effect of electrolytes, notably, sodium and chloride. Cell membranes are impermeable to these small ions. They influence water movement across the membranes to maintain isotonicity. The administration of hypotonic fluids causes cells to swell, whereas the administration of hypertonic fluids causes cells to shrink. Primary sodium depletion, vomiting, or overuse of diuretics may result in decreased extracellular volume, hypo-osmotic dehydration. Inappropriate secretion of antidiuretic hormone (ADH) may lead to enhanced water retention and hyponatremia, hypo-osmotic overhydration. Primary water loss, inadequate ADH secretion (diabetes insipidus), or lack of renal response to ADH (nephrogenic diabetes insipidus) results in hyperosmolarity caused by increased concentration of sodium ions in the circulation, hyperosmotic dehydration.

The balance of hydrostatic pressure and the colloid osmotic effect primarily determine the relative amounts of extracellular fluid distributed between plasma and the interstitium. The distribution of fluid between the

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intracellular and extracellular compartments is predominantly due to the osmotic effect of the electrolytes, notably sodium and chloride. Cell membranes are relatively impermeable to these ions, but water rapidly moves across so that the intracellular fluid remains isotonic relative to extracellular fluid. The rate of water diffusion is the rate of osmosis and is affected by disease and the parenteral administration of fluids. Relatively small changes in concentration of impermeable solutes in the extracellular fluid can cause marked changes in cell volume. Edema is the accumulation of an excessive amount of watery fluid in tissues or cells. Intracellular edema can occur in inflamed tissues and in tissues in which blood flow is reduced. Sodium ions that normally enter a cell during metabolic activity no longer can be effectively pumped out as a result of altered membrane function or permeability. The excess intracellular sodium causes osmosis of water into the cell, which causes the tissue to swell. A hypertonic extracellular environment causes a cell to shrink, whereas cells bathed in a hypotonic fluid will tend to swell. Because the brain is encased in bone, relatively small changes in cell volume can cause neurologic signs as an initial indication of altered sodium-fluid homeostasis.

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Abnormal leakage of fluid from the plasma to the interstitial spaces across capillaries or failure of the lymphatics to return fluid from the interstitium to the blood can result in extracellular edema. Dependent tissues tend to initially develop edema. Accelerated loss of water across the capillary wall can be due to altered permeability of the vessel, decreased colloid osmotic pressure, or increased hydrostatic pressure. Examples of each of these disturbances are, respectively, inflammation/hypersensitivity reactions, severe hypoalbuminemia, and heart failure. Blockage of lymphatic vessels (e.g., caused by intravessel cancer, mass effect compressing lymph vessels, or congenital absence) is the other major cause of extracellular edema. When edema fluid collects in a “potential” body space such as pleural, peritoneal, pericardial, or joint cavities or bursa, it is referred to as an *effusion*. The membranes lining these spaces do not offer effective resistance to the passage of fluids, electrolytes, or proteins, which move back and forth between the “potential” space and the interstitial fluid in the adjacent tissue. An effusion can develop as a result of disease involving organs within the space or the surrounding tissues. Conversely, because of the promiscuous nature of the lining membrane, the peritoneal cavity may be used for peritoneal dialysis to remove uremic substances and for the administration of isotonic fluids and drugs.

## 15.1.1 Alterations in Electrolytes (Fig. 14-2; see Figs. 13-2, 13-4, 14-1; Boxes 14-1 to 14-5; see Table 12-1)

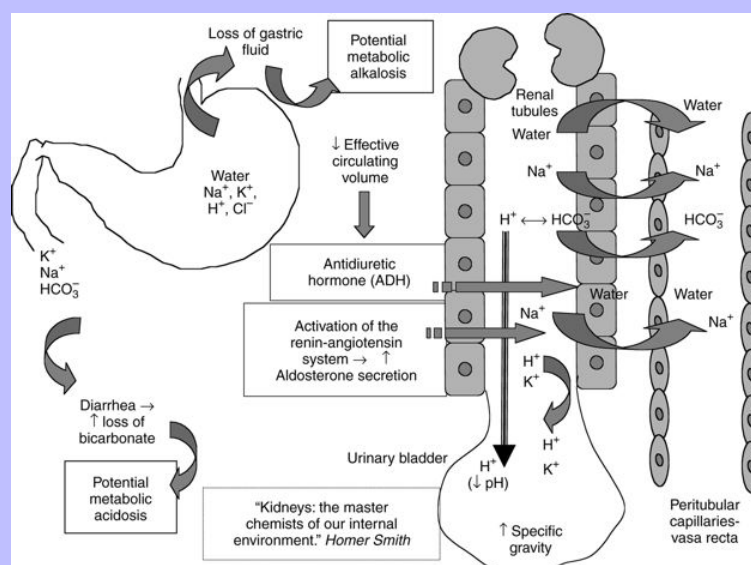
An abnormally low concentration of sodium ions in the circulation, hyponatremia, may be a consequence of sodium chloride loss from the extracellular fluid or the addition of excess water. Vomiting, diarrhea, certain diuretics, and insufficient aldosterone can accelerate the loss of sodium. Hypo-osmotic dehydration is generally the consequence. Excessive secretion of antidiuretic hormone may accelerate renal tension of water, resulting in overhydration and hyponatremia (hypo-osmotic overhydration). Hypernatremia, an abnormally high concentration of sodium ions in the circulation, may be a consequence of water loss from the extracellular fluid. Insufficient secretion of antidiuretic hormone, diabetes insipidus, or inadequate renal response to adequate levels of the hormone (nephrogenic diabetes insipidus) results in excess renal loss of water. Hypernatremia caused by loss of water causes hyperosmotic dehydration. Ingestion of salt supplements without access to adequate water may result in hypernatremia.

The extracellular potassium concentration is closely regulated as a result of the exquisite sensitivity of cell function following alterations. A seemingly minimal increase can cause life-threatening cardiac arrhythmias. The kidney, in concert with the secretion of aldosterone by the adrenal glands, has a pivotal role in potassium balance. Consequently, renal failure and hypoadrenocorticism (hypoadosteronism) are important causes of hyperkalemia, a greater than normal concentration of potassium ions in the circulation. Redistribution of potassium between the abundant intracellular compartment and the extracellular fluid space can also occur rapidly as a compensatory response or as a consequence of disease. Insulin is one of the most important

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physiologic factors that increase cell uptake of potassium. Acid-base abnormalities alter the intracellular-extracellular balance. Metabolic acidosis increases the efflux of potassium from the cell, and metabolic alkalosis has the opposite effect. Altered extracellular fluid osmolarity can cause redistribution of potassium from the intracellular compartment. Hyperglycemia caused by diabetes mellitus raises the extracellular osmolarity, causing cell dehydration and an attendant movement of potassium from the cells into the extracellular compartment. Factitious hyperkalemia (pseudohyperkalemia) may occur as a result of a delay in separating serum from blood with extreme leukocytosis or thrombocytosis because leukocytes and platelets are rich in potassium. The Akita dog has more potassium in its erythrocytes than other breeds. Delayed separation of serum from a clotted specimen may result in an increased potassium value. Horse and cattle erythrocytes are rich in potassium, and hemolysis or delayed separation of serum from a clotted specimen may result in an increased potassium value.

Fig. 14-2



Disorders of the gastrointestinal tract are common causes of acid-base/electrolyte disturbances. Gastric fluid is composed of water and high concentrations of hydrogen ( $\text{H}^+$ ) and chloride ( $\text{Cl}^-$ ) ions with lesser concentrations of sodium ( $\text{Na}^+$ ) and potassium ( $\text{K}^+$ ) ions, whereas intestinal fluid has prominent potassium and bicarbonate ions. Vomiting or sequestration of fluid in the ruminant abomasum may result in metabolic alkalosis. The reduction of the effective circulating blood volume and chloride and potassium losses are major factors that contribute to the maintenance of metabolic alkalosis. The kidney responds by reabsorbing water through accelerated release of antidiuretic hormone (ADH), and, most importantly, sodium reabsorption. Normally, chloride is the exchange anion with sodium in the proximal tubule, but because of its depletion, bicarbonate reabsorption is favored. In the distal tubule, sodium is normally exchanged with either hydrogen or potassium. As a result of potassium depletion, hydrogen exchange is increased by aldosterone. Renal retention of bicarbonate and accelerated hydrogen ion exchange result in additional loss of hydrogen ions in urine. Aciduria, an acid-base paradox, is the consequence. In contrast, the temporary increase in meal-induced acid secretion results in an excess of bicarbonate in the circulation, referred to as *an alkaline tide*. The physiologic renal response is to accelerate bicarbonate excretion, resulting in alkaline urine.

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An abnormally low concentration of potassium ions in the circulation, hypokalemia, may be a consequence of decreased intake, excessive loss, extracellular to intracellular shift, or dilution. Gastrointestinal loss is an important cause of accelerated potassium loss that may be exacerbated by inappetence. The concurrent loss of chloride in the vomitus or in sequestered abomasal fluid may produce hypokalemic, hypochloremic metabolic alkalosis. Excess loss will also occur as a result of hyperaldosteronism (hypernatremia often concurrent), renal tubular acidosis (hyperchloremic acidosis often concurrent), and the use of certain diuretics. The administration of furosemide to horses results in sufficient total body cation depletion to cause the formation of echinocytes. Insulin treatment in diabetic animals may reduce the circulating potassium level by promoting its cell uptake along with glucose. *Pseudohyperchloremia* refers to the artificially increased serum chloride value measured in epileptic animals treated with potassium bromide as an anticonvulsant.

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## 15.1.1.1 BOX 14-1 Causes of Hyperkalemia

Aldosterone deficiency (hypoadrenocorticism)

Urethral obstruction

Ruptured urinary bladder

Anuric or oliguric renal disease

Diffuse tissue necrosis

Hyperkalemic periodic paralysis (horse)

Delayed separation of clotted blood caused by high erythrocyte potassium concentration in most domestic species except cat and dog (exceptions are Akita and English springer spaniel breeds)

Collection of sample in potassium heparin

Metabolic acidosis

## 15.1.1.2 BOX 14-2 Causes of Hypokalemia

Gastrointestinal loss (vomiting, diarrhea)

Chronic renal disease or diet-related nephropathy (cats)

Postobstructive diuresis

Intravenous administration of potassium-poor fluids

Excessive diuretic treatment

Hypokalemic periodic paralysis

Hypokalemia syndrome in dairy cows

Administration of parenteral nutrition solutions

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Hyperaldosteronism

Renal tubular acidosis

Diabetes mellitus (in concert with insulin treatment)

### 15.1.1.3 BOX 14-3 Causes of Hyponatremia

Excessive sweating

Hyperglycemia (diabetes mellitus)

Chronic renal disease ("salt-losing" nephropathy)

Repeated drainage of pleural effusion

Sequestration of fluid or "third space" loss (pancreatitis, peritonitis, ruptured urinary bladder)

Congestive heart failure (edema)

Aldosterone deficiency (hypoadrenocorticism)

Excessive use of diuretics

Psychogenic polydipsia

Excessive (inappropriate) secretion of antidiuretic hormone (ADH)

### 15.1.1.4 BOX 14-4 Causes of Hypernatremia

Gastrointestinal loss (vomiting, diarrhea)

Inadequate intake (water deprivation)

Diabetes insipidus, "nephrogenic" diabetes insipidus

Increased insensible water loss (fever, high environmental temperature, panting)

Renal disease, postobstructive diuresis

Diabetes mellitus (after insulin treatment)

Osmotic diuresis

Excessive salt ingestion (salt poisoning) or intravenous administration of hypertonic saline solution

Hyperaldosteronism

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## 15.1.1.5 BOX 14-5 Causes of Altered Anion Gap

### 15.1.1.5.1 Increased

Acidosis: Lactic acidosis, ethylene glycol toxicosis, uremia, diabetic ketoacidosis, grain overload, ketosis, strenuous exercise, salicylate toxicosis

Necrotizing enterocolitis in horses

Administration of carbonic anhydrase inhibitor

Renal tubular acidosis

### 15.1.1.5.2 Decreased

Hypoalbuminemia

Polyclonal gammopathy

The difference or gap between the circulating concentrations of sodium, the major cation, and the major anions, chloride and bicarbonate, is relatively consistent within species. It represents the amount of unmeasured anions in the circulation, primarily negatively charged proteins, which are not normally measured by conventional techniques. Potassium has been used in the calculation of the anion gap but adds no interpretative value. If it is used, the reference ranges for the anion gap will be slightly higher. Changes in the anion gap may help sort out the possible causes of acid-base disturbances. An increased anion gap, in concert with metabolic acidosis, suggests an increase in metabolizable or nonmetabolizable acid levels in the circulation. An increase in the lactic acid level may be due to hypovolemic shock, strenuous exercise, or grain overload. An enhanced production of ketone bodies, ketoacidosis, may be associated with diabetes mellitus or ketosis. The retention of sulfates and phosphates (nonmetabolizable acids) caused by renal failure contributes to uremic acidosis. Toxicosis caused by ethylene glycol, salicylate, paraldehyde, or methanol (nonmetabolizable acids) may result in acidosis and increase the anion gap. A decreased anion gap without an acid-base disturbance may be due to a gammopathy or hypoalbuminemia. A metabolic acidosis with hyperchloremia and a normal to decreased anion gap may develop as a result of diarrhea-induced bicarbonate loss or renal tubular acidosis. In addition, these disorders may be associated with a potassium reduction. In contrast, the potassium value is often increased in association with a hyperchloremic metabolic acidosis with a normal to decreased anion gap caused by aldosterone deficiency, hypoadrenocorticism.

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Electrolytes in the urine can be assessed by calculating their fractional excretion rate (FE).

$$FE (\%) = \frac{[\text{Urine electrolyte (mEq/L)} \div \text{Serum electrolyte (mEq/L)}] \times [\text{Serum creatinine (mg/dL)} \div \text{Urine creatinine (mg/dL)}] \times 100}{100}$$

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Table 14-1 Characteristics of Primary Acid-Base Disturbances

	pH	[H <sup>+</sup> ]	[HCO <sub>3</sub> ]	PCO <sub>2</sub>
Metabolic alkalosis	↑	↓	↑	↑
Metabolic acidosis	↓	↑	↓	↓
Respiratory acidosis	↓	↑	↑	↑
Respiratory alkalosis	↑	↓	↓	↓
Gastrointestinal loss (vomiting, diarrhea)	↑	↓	↓	↓
Primary events are indicated by double arrows.				

## 15.2 ACID-BASE (See Fig. 14-2; Table 14-1; Boxes 14-6 to 14-9)

Most of the hydrogen ion concentration in the circulation arises from the metabolism of glucose, other carbohydrates, and weak acids. The concentration of hydrogen ions in a fluid is determined by measuring its pH, a value that varies inversely with the hydrogen ion concentration. Bicarbonate ions buffer excess hydrogen ions forming bicarbonate which causes increased carbon dioxide and water production through the action of carbonic anhydrase located in the lung alveoli and renal tubular epithelial cells. The excess carbon dioxide stimulates respiration resulting in its elimination and the carbon dioxide reacts with water in renal tubules to form bicarbonate. Acid-base disturbances are defined by determining whether the principal change primarily involves bicarbonate or carbon dioxide. Metabolic acidosis and respiratory acidosis are defined by a reduction in the pH caused by a prominent reduction of bicarbonate ions and a prominent increase in carbon dioxide, respectively. Conversely, a rise in the pH caused by a prominent increase in bicarbonate ions and a prominent reduction of carbon dioxide are classified as metabolic alkalosis and respiratory alkalosis, respectively. The kidneys or lungs generally try to correct the primary acid-base disturbance, but the response is seldom complete in clinical disorders. The resulting compensated bicarbonate or carbon dioxide changes are generally of a lesser magnitude.

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### 15.2.1 BOX 14-6 Causes of Metabolic Acidosis

Lactic acidosis (shock, strenuous exercise)

Ethylene glycol toxicosis

Uremia

Hypoadrenocorticism

Diabetic ketoacidosis

Ketosis

Grain overload

Necrotizing enterocolitis in horses

Salicylate toxicosis

Paraldehyde toxicosis

Diarrhea

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Overuse of carbonic anhydrase inhibitors

Renal tubular acidosis

Oral acidifying agents

### 15.2.2 BOX 14-7 Causes of Metabolic Alkalosis

Vomiting

Sequestration of fluid in abomasum or forestomach

Overuse of loop diuretics, thiazide

Excess administration of sodium bicarbonate

Excessive sweat loss in horses

Primary hyperaldosteronism

### 15.2.3 BOX 14-8 Causes of Respiratory Acidosis

Cardiopulmonary arrest

Impaired pulmonary function: Airway obstruction, pulmonary parenchymal disease, pneumothorax

Anesthesia, central nervous system depressant drugs

### 15.2.4 BOX 14-9 Causes of Respiratory Alkalosis

Hypoxemia: Congestive heart failure, right-to-left shunting, severe anemia

Hyperventilation: Central nervous system stimulation—drugs (salicylate toxicosis, xanthine toxicosis), pain, gram-negative sepsis

Excessive mechanical ventilation

### 15.3 ADDITIONAL READING\*

HS Autran de Morais, DJ Chew: Use and interpretation of serum and urine electrolytes. *Semin Vet Med Surg.* 7, 1992, 262–274.

\*PD Constable: Clinical assessment of acid-base status: Comparison of the Henderson-Hasselbalch and strong ion approaches. *Vet Clin Pathol.* 29, 2000, 115–128.

\*PD Constable, KW Hinchcliff, Muir, WW III : Comparison of anion gap and strong ion gap as predictors of unmeasured strong ion concentration in plasma and serum of horses. *Am J Vet Res.* 59, 1998, 881–887.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- \*PD Constable, RN Streeter, Koenig, GJ, et al.: Determinants and utility of the anion gap in predicating hyperlactemia in cattle. *J Vet Intern Med.* **11**, 1997, 71–79.
- SP DiBartola, SE Johnson, SJ Davenport, et al.: Clinicopathologic findings resembling hypoadrenocorticism in dogs with primary gastrointestinal disease. *J Am Vet Med Assoc.* **187**, 1985, 60–63.
- SW Dow, MJ Fettman: Chronic renal disease and potassium depletion in cats. *Semin Vet Med Surg.* **7**, 1992, 198–201.
- \*FJ Gennari: Hypokalemia. *N Engl J Med.* **339**, 1998, 451–458.
- \*SL Gluck: Acid-base. *Lancet.* **352**, 1998, 474–479.
- TK Graves, WD Schall, K Refsal, et al.: Basal and ACTH-simulated plasma aldosterone concentrations are normal or increased in dogs with trichuriasis-associated pseudohypoadrenocorticism. *J Vet Intern Med.* **8**, 1994, 287–289.
- S Greenberg, IW Reiser, SY Chou, et al.: Trimethoprim-sulfamethoxazole induces reversible hyperkalemia. *Ann Intern Med.* **119**, 1993, 291–296.
- \*VL Hood, RL Tannen: Protection of acid-base balance by pH regulation of acid production. *N Engl J Med.* **339**, 1998, 819–826.
- \*D Hughes, ER Rozanski, FS Shofer, et al.: Effect of sampling site, repeated sampling, pH, and Pco<sub>2</sub> on plasma lactate concentration in healthy dogs. *Am J Vet Res.* **60**, 1999, 521–524.
- \*LR Johnson, MR Lappin, DC Baker: Pulmonary thromboembolism in 29 dogs: 1985-1995. *J Vet Intern Med.* **13**, 1999, 338–345.
- \*PA Jones, FT Bain, TD Byars, et al.: Effect of hydroxyethyl starch infusion on colloid oncotic pressure in hypoproteinemic horses. *Am J Vet Res.* **218**, 2001, 1130–1135.
- \*S Kumar, T Berl: Sodium. *Lancet.* **352**, 1998, 220–226.
- \*AL Leisewitz, LS Jacobson, de Morais, HAS, et al.: The mixed acid-base disturbances of severe canine babesiosis. *J Vet Intern Med.* **15**, 2001, 445–452.
- PJ Pascoe, JE Ilkiw, PH Kass, et al.: Case-control study of the association between intraoperative administration of nafcillin and acute postoperative development of azotemia. *J Am Vet Med Assoc.* **208**, 1996, 1043–1047.
- \*G Perkins, SJ Valberg, JM Madigan, et al.: Electrolyte disturbances in foals with severe rhabdomyolysis. *J Vet Intern Med.* **12**, 1998, 173–177.
- RM Rault: Case report: Hyponatremia associated with nonsteroidal antiinflammatory drugs. *Am J Med Sci.* **305**, 1993, 318–320.
- \*KA Reimann, GG Knowlen, HW Tvedten: Factitious hyperkalemia in dogs with thrombocytosis. *J Vet Intern Med.* **3**, 1989, 47–52.
- \*DT Runk, JE Madigan, CJ Rahal, et al.: Measurement of plasma colloid osmotic pressure in normal thoroughbred neonatal foals. *J Vet Intern Med.* **14**, 2000, 475–478.
- \*WJ Saville, KW Hinchcliff, BR Moore, et al.: Necrotizing enterocolitis in horses: A retrospective study. *J Vet Intern Med.* **10**, 1996, 265–270.
- \*ES Sielman, RW Sweeney, RH Whitlock, et al.: Hypokalemia syndrome in dairy cows: 10 cases (1992-1996). *J Am Vet Med Assoc.* **210**, 1997, 240–243.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

SJ Spier, GP Carlson, D Harrold, et al.: Genetic study of hyperkalemic periodic paralysis in horses. <i>J Am Vet Med Assoc.</i> <b>202</b> , 1993, 933–938.	243
*RH Stewart: The case for measuring plasma colloid osmotic pressure. <i>J Vet Intern Med.</i> <b>14</b> , 2000, 473–474.	244
H Valazquez, MA Perazella, FS Wright, et al.: Renal mechanism of trimethoprim-induced hyperkalemia. <i>Ann Intern Med.</i> <b>119</b> , 1993, 296–301.	
*IK van Vonderen, HS Kooistra, Sprang, EPM, et al.: Disturbed vasopressin release in 4 dogs with so-called primary polydipsia. <i>J Vet Intern Med.</i> <b>13</b> , 1999, 419–425.	
DJ Weiss, R Geor, CM Smith, et al.: Furosemide-induced electrolyte depletion associated with echinocytosis in horses. <i>Am J Vet Res.</i> <b>53</b> , 1992, 1769–1772.	
*KJ Whitehair, SC Haskins, JG Whitehair, et al.: Clinical applications of quantitative acid-base chemistry. <i>J Vet Intern Med.</i> <b>9</b> , 1995, 1–11.	
MD Willard, TW Fossum, A Torrence, et al.: Hyponatremia and hyperkalemia associated with idiopathic or experimentally induced chylothorax in four dogs. <i>J Am Vet Med Assoc.</i> <b>199</b> , 1991, 353–358.	
* New references added to the third edition.	

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## 16 Chapter 15 Evaluation of Fluids: Effusions, Synovial Fluid, Cerebrospinal Fluid

### 16.1 EVALUATION OF EFFUSIONS

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Collection of fluid from a body cavity is usually done by using a 20- to 22-gauge needle and a 10- to 12-mL syringe. After surgical preparation of the skin, the needle with syringe attached is inserted into the subcutaneous tissue, and slight negative pressure is applied as the needle is advanced at an angle. Fluid will appear in the syringe as soon as the needle penetrates the lining of the cavity. Angling the needle helps prevent fluid from leaking from the cavity into the surrounding tissue. A small drop of fluid is placed on a glass slide near the frosted end and thinly layered. The spreader slide is stopped before going off the end of the glass slide and the excess fluid is allowed to flow partially back toward the frosted end. Drying of the slide preparation is facilitated by a warm air blower. Such a two-part slide provides for an estimate of the cell count from the thin area, and the concentrated area can be searched for cell clumps or organisms. The remaining fluid should be placed in an ethylenediaminetetraacetic acid (EDTA) tube (purple top) to prevent any potential clotting, and the fluid can be used for cell counts and measurement of other biochemical values that may need to be determined at a later date. Evaluation of the cell component includes an absolute and differential nucleated cell count. Fluids with very low cell counts are concentrated by routine centrifugation or with a cytocentrifuge.

### 16.2 TRANSUDATE, MODIFIED TRANSUDATE, AND EXUDATE (Table 15-1, Fig. 15-1)

*When the liver is full of fluid and this overflows into the peritoneal cavity, so that the belly becomes full of water, death follows. Hippocratic aphorism.*

**From Atkinson M: Ascites in liver disease. Postgrad Med 1956;32:482-494.**

#### 16.2.1 Transudate and Modified Transudate

A transudate is principally classified by a protein concentration that is less than 2.5 g/dL, and a modified transudate, by a protein concentration greater than 2.5 g/dL. Transudates and modified transudates generally have low nucleated cell counts that are predominantly composed of mesothelial cells. Albumin is exclusively synthesized by hepatocytes and traverses the lymph-filled spaces of Disse and the porous endothelium into the hepatic sinusoids for delivery into the systemic circulation. The protein content of hepatic lymph approximates 90% of the plasma total protein concentration. Increased hepatic venous pressure caused by right-sided heart failure or compression of the caudal vena cava results in leakage of protein-rich hepatic lymph into the abdomen and contributes to the formation of a protein-rich ascites classified as a modified transudate. The protein content of lymph is low in other organs. Consequently, the leakage of low-protein-containing lymph from the intestines caused by portal hypertension forms a low-protein fluid classified as a transudate. Portal vein hypoplasia and hepatic cirrhosis are causes of portal hypertension. A reduction in the circulating albumin level of 1.0 g/dL or less can be a primary cause of an ascitic transudate. Concurrent dependent edema may be present as a result of the markedly reduced oncotic pressure.

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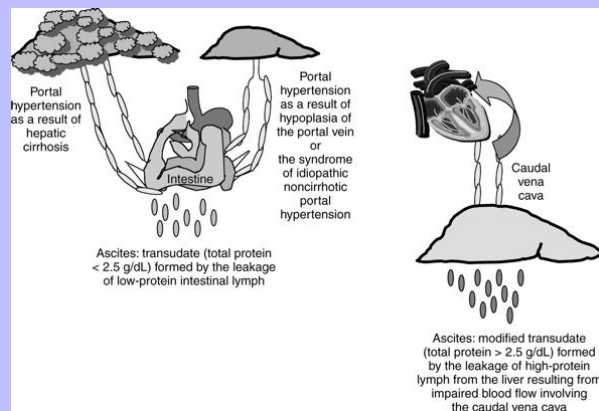
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Table 15-1 Guidelines for Characterizing Effusions Other than Hemorrhagic Effusions

Finding	Category		
	Transudate	Modified Transudate	Exudate
Total protein (g/dL)	<2.5	>2.5	>2.5
Nucleated cell count (/μL)	<1000	<1000	>5000
	<5000 (Horse)	<5000 (Horse)	>10,000 (Horse)
Predominant nucleated cell type	Mesothelial/macrophage Horse: up to 60% may be nondegenerate neutrophils	Mesothelial/macrophage Horse: up to 60% may be nondegenerate neutrophils	Neutrophil
Common Causes			
	Portal hypertension secondary to hepatic insufficiency or portal vein hypoplasia	Right-sided heart failure Impaired venous flow between origin of hepatic vein and right atrium of the heart	Inflammation: septic Inflammation: nonseptic—feline infectious peritonitis irritant: urine, bile, chyle, foreign body
	Space-occupying mass Severe hypoalbuminemia (serum albumin <~1.5 g/dL)	Space-occupying mass Horse: intestinal disorder	Space-occupying mass Horse: intestinal disorder

Fig. 15-1



Albumin is exclusively synthesized by hepatocytes and traverses the lymph-filled spaces of Disse and the porous endothelium into the hepatic sinusoids for delivery into the systemic circulation. The protein content of hepatic lymph approximates 90% of the plasma total protein concentration. Increased hepatic venous pressure (e.g., caused by right-sided heart failure) causes leakage of protein-rich hepatic lymph into the abdomen and contributes to the formation of a protein-rich ascites classified as a modified transudate. The protein content of lymph is low in other organs. Consequently, the leakage of low-protein—containing lymph from the intestines caused by portal hypertension forms a low-protein fluid classified as a transudate.

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## 16.2.2 Exudate

An exudate is principally characterized by an increased number of nucleated cells consisting primarily of neutrophils. A protein concentration greater than 3.0 g/dL is concurrent as a result of the vascular leakage of protein associated with the inflammatory process. Macrophages and lymphocytes are often observed, especially with chronicity. Infectious and noninfectious inflammatory causes of an exudate can usually be distinguished by microscopic examination and biochemical determinations. Septic exudates are defined by the identification of bacteria in the fluid (Plate 20). The presence of bacteria is one cause of neutrophil karyolysis (swelling of the nucleus and reduced staining intensity). Another cause of neutrophil karyolysis in a sterile exudate in the abdomen is the release of proteolytic enzymes as a result of acute pancreatitis. The lipase level in the ascitic fluid is usually greater than the lipase level in the circulation. Feline infectious peritonitis (FIP) is one example in which a sterile exudate can involve the pleural or peritoneal cavities (Plate 21). This exudate has a notably high protein concentration that may approximate the protein level in the circulation. Globulins constitute a significant component of the total protein content. Electrophoresis of FIP-related effusions often reveals an albumin to globulin ratio of less than 0.81. Irritants may also cause formation of a sterile exudate. Examples include bile from a ruptured gallbladder and urine from a ruptured urinary bladder. Pigment-filled macrophages and a bilirubin level that is higher in the abdominal fluid than in the circulation are usually associated with a ruptured gallbladder. A creatinine level that is higher in the abdominal fluid than the creatinine level in the circulation is usually an initial sequela of a ruptured urinary bladder.

## 16.2.3 Equine Abdominal Fluid

The normal equine abdominal fluid has a total nucleated cell count of approximately 5000 to 9000/mL. In contrast to the dog and cat, the principal nucleated cell type is the neutrophil. Neutrophils may represent up to 60% of the total nucleated cells. The normal protein concentration is less than 2.5 g/dL. A value greater than 2.5 g/dL is suggestive of abdominal disease.

## 16.2.4 Chylous Effusion

Chyle is a turbid white or pale-yellow fluid taken up by lacteals from the intestine during digestion and carried by the lymphatic system via the thoracic duct into the venous circulation. The milky appearance is due to the high chylomicron content in the lymph. A chylous effusion may develop in the pleural or peritoneal cavity as a result of a variety of diseases. Because of the high concentration of triglyceride-rich chylomicrons in chyle, a triglyceride level in the fluid that is higher than the triglyceride level in the circulation is indicative of a chylous effusion. Obtaining a specimen soon after a meal will accentuate the difference if the effusion has a chylous component. A low total cell number consisting of small to medium-sized lymphocytes is initially observed by microscopic examination. Chyle is an irritant and prompts the influx of neutrophils, resulting in the formation of a sterile exudate. Lesser numbers of macrophages and reactive mesothelial cells may be admixed. The refractometer-determined total protein level will be increased because of the presence of chylomicrons. The microscopic identification of neoplastic cells, notably immature lymphocytes, defines cancer as the underlying cause of its formation. Staining with Sudan black or oil red O and ether clearance procedures are unreliable tests for the detection of chylomicrons.

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## 16.2.5 Neoplastic Effusions

The neoplastic cell types most commonly associated with pleural or peritoneal effusions are malignant lymphoma and carcinomas (Plate 30) because of their ease of exfoliation. Reactive mesothelial cells may mimic carcinoma cells and even form structures suggestive of adenocarcinoma. Their differentiation from cancer cells may be problematic.

## 16.2.6 Pericardial Effusions

The two more common causes of pericardial effusions in the dog are idiopathic (hemorrhagic) mechanisms and neoplasia (hemangiosarcoma and heart-base tumor). The German shepherd and golden retriever breeds appear to have an increased incidence of hemangiosarcoma, and brachycephalic breeds have a higher incidence of heart-base tumor. An increased incidence of idiopathic pericardial effusion appears to be associated with great Pyrenees, great Dane, Saint Bernard, and golden retriever breeds. The cytologic and biochemical evaluation of a pericardial effusion has limited value in determining its cause. Blood is an irritant, and the exfoliation of reactive mesothelial cells into the pericardial effusion may mimic neoplastic cells and confound the differential diagnosis. Determination of the effusion's pH by using a urinal-ysis reagent strip appears to be diagnostically informative. A pH greater than 7.0 is supportive of neoplasia, whereas a pH less than 7.0 is more commonly associated with nonneo-plastic disease.

## 16.3 EVALUATION OF SYNOVIAL FLUIDS

*Another source of fallacy is the vicious circle of illusions which consist on the one hand of believing what we see and, on the other, seeing what we believe.*

**Sir Clifford Allbutt**

Indications for obtaining synovial fluid include the following: (1) a swollen hot joint suggestive of an infective process; (2) radiographic findings suggestive of a degenerative joint process (a synovial fluid analysis is obtained to rule out a concomitant inflammatory process); and (3) idiopathic polyarthritis in which the patient has a history of nondescript pain or rotating leg lameness.

Synovial fluid is obtained by using a 23- to 25-gauge needle attached to a 3-mL syringe. The area over the joint is surgically prepared, the needle is inserted into the joint space, and synovial fluid is gently aspirated. One or two drops are adequate if there is a limited amount. Aggressive sampling can easily contaminate the specimen with blood. Aspiration is terminated, the needle is removed, and the fluid is placed in an EDTA tube. If only 1 or 2 drops are obtained, slides can be directly made. Cytologic preparation is the same as that for blood films, except that the spreader slide is moved slowly away from the drop to facilitate a protracted thin feathering of the fluid. Cell structure is obscured in thick specimens. If possible, several drops are placed in culture media or left in the sterile syringe, capped, and saved for possible bacterial culture if the cytologic examination suggests an infectious process.

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## 16.3.1 Cell Types: Normal and Abnormal Findings (Case 15-1)

Normal canine and feline synovial fluid has less than 3000 nucleated cells per microliter. Normal equine synovial fluid has less than 500 nucleated cells per microliter. Most of the nucleated cells are small to medium-sized mononuclear cells with less than 10% neutrophils. A granular eosinophilic background is often observed.

Hemarthrosis is associated with trauma or coagulation disorders, notably factor deficiency in young animals and warfarin intoxication. Synovial fluid associated with degenerative joint disease usually has a normal nucleated cell number but an increased proportion of foamy macrophages. Eosinophilic granules may be noted in the cytoplasm.

Increased numbers of neutrophils in the synovial fluid are associated with septic arthritis and nonseptic disease involving the joint. Septic exudates are defined by the identification of bacteria in the fluid and generally involve one joint (Plate 20). The presence of bacteria is one cause of neutrophil karyolysis. An oil immersion objective is used to search for bacteria. Rickettsial or *Borrelia* infections can be included under both noninfectious and infectious categories because the neutrophilic inflammation may be directly caused by joint infection or may be an indirect manifestation of systemic disease. Rickettsial organisms can occasionally be observed in neutrophils. Serologic testing is warranted when septic arthritis is not present.

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Nonseptic, steroid-responsive arthritis often involves multiple joints and is idiopathic. The antibacterial trimethoprim-sulfamethoxazole is one known case of nonseptic polyarthritis. Nonseptic arthritis is one manifestation of systemic lupus erythematosus. Patients with symptoms suggestive of lupus can be screened by use of an antinuclear antibody assay. A strongly positive antinuclear antibody titer is supportive of lupus. Lupus erythematosus cells may be found in the synovial fluid of patients with systemic lupus erythematosus. The lupus erythematosus cell is a neutrophil that contains a large amorphous clump of protein.

## 16.4 EVALUATION OF CEREBROSPINAL FLUID

*Science ... such wholesome returns of conjecture out of such trifling bits of facts.*

***Life on the Mississippi*, Mark Twain, pen name of Samuel Longhorn Clemens, 1835-1910**

Cerebrospinal fluid (CSF) is a clear colorless ultrafiltrate of the plasma secreted by the choroid plexuses of the ventricles of the brain, filling the ventricles and the subarachnoid cavities of the brain and spinal cord. Pathologic changes in the outer surfaces of these structures may cause changes in the CSF, whereas pathologic changes deeper in the nervous tissue usually do not. Evaluation of the CSF routinely includes a nucleated cell count, cytologic examination, and total protein determination. Other biochemical measurements that can be performed include determination of glucose, creatine kinase, and lactic dehydrogenase levels; protein electrophoresis; and specific immunoglobulin quantification. Turbidity implies an increased number of nucleated cells (pleocytosis). A pink to red color indicates the presence of erythrocytes, and a yellow discoloration (xanthochromia) suggests prior hemorrhage in the patient without icterus.

The normal CSF has less than three to five nucleated cells per microliter and sparse erythrocytes. A hemocytometer chamber is used to perform cell counts. The chamber is composed of nine large (1-mm<sup>2</sup>) squares. The numbers of nucleated cells or erythrocytes in all nine large squares are counted, added together, and

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multiplied by 10/9 to give a value per microliter. The condenser of the microscope is lowered to enhance the silhouettes of the cells and aid in differentiating erythrocytes (anuclear, crenated) from nucleated cells. Nucleated cells rapidly deteriorate on standing. Refrigeration of the specimen will delay loss of cell structure for several hours. The use of a cytocentrifuge provides a cell preparation of uniform quality with good preservation of cell structure.

Small to medium mononuclear cells consisting of monocytoïd cells and lymphocytes are normal microscopic findings in the CSF. Immunophenotyping of mononuclear cells in canine CSF indicates that the mean proportion of CD4<sup>+</sup> and CD21<sup>+</sup> cells is significantly lower in CSF compared with that in blood. The mean proportion of CD14<sup>+</sup> and CD8α<sup>+</sup> cells is similar for CSF and blood. A neutrophilic pleocytosis is associated with bacterial infection, sterile-steroid-responsive disease (notably in young adult dogs), and meningiomas. A mixture of neutrophils, lymphocytes, and macrophages may be associated with feline infectious peritonitis, granulomatous meningoencephalomyelitis, and infection with *Neospora caninum*. Mononuclear pleocytosis with lymphocytic prominence and increased protein concentration is most likely to be observed in horses with West Nile virus infection. The abnormal findings are more likely to be present in the lumbosacral sample rather than in a specimen obtained from the atlanto-occipital area. Equine protozoal myeloencephalitis is an infectious disease of the central nervous system in horses that is usually caused by *Sarcocystis neurona*. It is diagnosed by demonstrating the presence of specific anti-*Sarcocystis neurona* antibody in the CSF by immunoblot testing. The test has low sensitivity and specificity, but a negative result supports the lack of infection with *Sarcocystis neurona*. Routine evaluation of the CSF is performed to detect other neurologic disease such as West Nile viral infection and to determine the erythrocyte count before submission of a sample for immunoblot testing. Because of the potential for a high concentration of anti-*Sarcocystis neurona* antibodies in the circulation, blood contamination during CSF collection may cause a false-positive immunoblot result. A CSF specimen with less than 5 erythrocytes per microliter is recommended for immunoblot testing. A prominent eosinophilic pleocytosis has been described in young adult dogs, with an apparent increased incidence in the golden retriever. The large yeast forms of cryptococcosis are readily identified with routine stains. Malignant lymphoma is the most common neoplasm of the central nervous system diagnosed by cytologic examination of the CSF. Occasionally, small clumps of epithelium-like cells will exfoliate from the choroid plexuses and ependymal lining.

### 16.4.1 Protein and Glucose

Quantification of the total protein concentration requires a dye-binding or turbidimetric procedure because of the low (only milligram) concentrations in the CSF. The protein concentration is less than 30 mg/dL. A urinalysis reagent strip may be used to obtain an initial estimate of the protein concentration before the sample is submitted to a laboratory for quantification. An increased protein level is usually indicated by positive reagent strip readings of 30 mg/dL or greater. The reagent strip may not detect a CSF protein level of less than 60 mg/dL. Increased protein concentrations are associated with a variety of diseases.

The glucose concentration of CSF is 60% to 80% that of plasma and is measured by the same method. A reduction in the CSF glucose concentration, hypoglycorrhachia, may be associated with hypoglycemia or bacterial meningitis.

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## 16.5 ADDITIONAL READING\*

### 16.5.1 Effusions

\*SE Bunch, SE Johnson, JM Cullen: Idiopathic noncirrhotic portal hypertension in dogs: 33 dogs (1982-1998). *J Am Vet Med Assoc.* **218**, 2001, 392–399.

NJ Edwards: The diagnostic value of pericardial fluid pH determination. *J Am Anim Hosp Assoc.* **32**, 1996, 63–67.

\*K Papasouliotis, K Murphy, S Dodkin, et al.: Use of the Vetest 8008 and refractometer for determination of total protein, albumin, and globulin concentrations in feline effusions. *Vet Clin Pathol.* **31**, 2002, 162–166.

### 16.5.2 Cerebrospinal Fluid

CS Bailey, RJ Higgins: Comparison of total white blood cell count and total protein content of lumbar and cisternal cerebrospinal fluid of healthy dogs. *Am J Vet Res.* **46**, 1985, 1161–1165.

CS Bailey, RJ Higgins: Characteristics of cisternal cerebrospinal fluid associated with primary brain tumors in the dog: A retrospective study. *J Am Vet Med Assoc.* **188**, 1986, 414–417.

\*C Duque, J Parent, D Bienzle: The immunophenotype of blood and cerebrospinal fluid mononuclear cells in dogs. *J Vet Intern Med.* **16**, 2002, 714–719.

\*MO Furr, H Bender: Cerebrospinal fluid variables in clinically normal foals from birth to 42 days of age. *Am J Vet Res.* **55**, 1994, 781–784.

M Furr, R MacKay, D Granstrom, et al.: Clinical diagnosis of equine protozoal myeloencephalitis (EPM). *J Vet Intern Med.* **16**, 2002, 618–621.

EM Green, GM Constantinescu, RA Kroll: Equine cerebrospinal fluid: Physiologic principles and collection techniques. *Contin Educ Pract Vet.* **14**, 1992, 229–238.

EM Jamison, JH Lumsden: Cerebrospinal fluid analysis in the dog. Methodology and interpretation. *Semin Vet Med Surg.* **3**, 1988, 122–132.

SB Lane, JN Kornegay, JR Duncan, et al.: Feline spinal lymphosarcoma: A retrospective evaluation of 23 cats. *J Vet Intern Med.* **8**, 1994, 99–104.

JS Rand, J Parent, R Jacobs, et al.: Reference intervals for feline cerebrospinal fluid: Cell counts and cytologic features. *Am J Vet Res.* **51**, 1990, 1044–1048.

JS Rand, J Parent, R Jacobs, et al.: Reference intervals for feline cerebrospinal fluid: Biochemical and serologic variables, IgG concentration, and electrophoretic fractionation. *Am J Vet Res.* **51**, 1990, 1049–1054.

JS Rand, J Parent, D Percy, et al.: Clinical, cerebrospinal fluid, and histological data from twenty-seven cats with primary inflammatory disease of the central nervous system. *Can Vet J.* **35**, 1994, 103–110.

JS Rand, J Parent, D Percy, et al.: Clinical, cerebrospinal fluid, and histological data from thirty-four cats with primary noninflammatory disease of the central nervous system. *Can Vet J.* **35**, 1994, 174–181.

LL Smith-Maxie, JP Parent, J Rand, et al.: Cerebrospinal fluid analysis and clinical outcome of eight dogs with eosinophilic meningoencephalitis. *J Vet Intern Med.* **3**, 1989, 167–174.

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M Vandeveld, JS Spano: Cerebrospinal fluid cytology in canine neurologic disease. *Am J Vet Res.* **38**, 1977, 1827–1832.

\*HL Wamsley, R Alleman, MB Porter, et al.: Findings in cerebrospinal fluids of horses infected with West Nile virus: 30 cases (2001). *J Am Vet Med Assoc.* **221**, 2002, 1303–1305.

EG Welles, JW Tyler, DC Sorjonen, et al.: Composition and analysis of cerebrospinal fluid in clinically normal adult cattle. *Am J Vet Res.* **53**, 1992, 2050–2056.

WR Widner, DB DeNicola, WE Blevens, et al.: Cerebrospinal fluid changes after iopamidol and metrizamide myelography in clinically normal dogs. *Am J Vet Res.* **53**, 1992, 396–401.

\* New references added to the third edition.

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## 17 Chapter 16 Management and Classification of Tissue Specimens Obtained with Fine-Needle Aspiration Biopsy

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DR. WATSON: "And yet I believe my eyes are as good as yours."

SHERLOCK HOLMES: "Quite so. You see but do not observe. The distinction is quite clear."

Arthur Conan Doyle,

*Scandal in Bohemia*

The microscopic examination of the cytologic specimen is a valuable, simple, pragmatic, and financially rewarding test in veterinary medicine. Fine-needle aspiration biopsy provides an opportunity for the aspirator to also be the interpreter. This maximizes the link between the historical information and findings on the physical examination and the interpretation of the cytologic specimen. It permits an immediate triage of the specimen to determine adequacy of the specimen and can provide immediate diagnostic information. The objective of this chapter is to discuss sample management and define the value and limitations of cytology for the differential diagnosis of inflammatory disorders and neoplasia. Colored photographs of sample management procedures and photomicrographs of the specimens discussed can be found in a separate atlas listed in Suggested Reading.

### 17.1 THE MICROSCOPE AND OBJECTIVES

The use of two microscopes is preferable in a clinical setting. A high-quality microscope is dedicated to the examination of "clean" (hematologic and cytologic) specimens, and another of lesser quality is used for the evaluation of "dirty" specimens (fecal parasite and skin scraping examinations). The "dirty" specimens can be adequately scanned with a 10× objective and examined more closely with a 20× objective. The latter will identify most infectious agents in these types of specimens. Planachromat objectives are recommended for use with the high-quality microscope. The combination of 20× and 50× oil objectives is "user friendly" for most clinical applications pertaining to hematology and cytology. They permit a meaningful scan of the slide and obviate the need for cover-slipping, which is required for the 40× (high-dry) objective. Inadvertent "dragging" of the 40× objective through the immersion oil is a common reason for an "out of focus" appearance of specimens and contributes to the early demise of the objective. The addition of a 100× (oil) objective amplifies the ability to visualize bacteria such as *Haemobartonella* and *Ehrlichia* species. The 20× objective facilitates an efficient determination of the differential leukocyte count on the blood film and the recognition of the predominant cell type comprising cytologic specimens. Problematic cell types can be subsequently investigated by the use of the higher-power objective. Microscopic alignment and adjustment of the condensing system should be done on an annual basis to maximize the optical value of the microscope.

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### 17.2 SPECIMEN MANAGEMENT

A quality cytologic specimen is essential for quality results. Acquisition and management of the specimen are skills that must be practiced. Hepatic and lymphoid issues exfoliate easily. These tissues, obtained from fresh necropsies, make excellent choices for practicing aspiration, touch-imprint, and slide preparation techniques.

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## 17.2.1 Obtaining the Specimen and Preparing the Slide

The full value of the microscopic examination of a cytologic specimen is realized when it is frequently used in the appropriate differential diagnostic process. In general, body cavity fluids, cutaneous and subcutaneous masses, lymph nodes, and diffuse organomegaly lend themselves to fine-needle aspiration biopsy.

Recommendations for needle and syringe sizes vary and usually reflect personal preference; each person should use what works best for him or her. A 20- to 22-gauge, 1-inch needle with a 6- to 12-mL syringe is a useful combination for obtaining many of these specimens. The needle and syringe can be connected with a silicone rubber (Silastic) tube, the type used for the administration of intravenous fluids, to provide greater flexibility and accommodate unexpected patient movement. A 2½-inch, 22-gauge spinal needle with stylet can be used for sampling internal organs. When a spinal needle is used, the stylet is removed after the organ of interest is penetrated, a syringe is attached, and suction is applied. As with other aspiration techniques, the suction is gently relieved before the needle is removed from the tissue.

The process of aspiration is not necessary for obtaining a cytologic specimen from certain tissues. A technique that is based on the principle of capillarity, referred to as *fine-needle capillary sampling*, is performed by placement of a needle without a syringe attached into the lesion. Cells are displaced into the cylinder of the needle by capillary action. The technique has been successfully applied to the diagnosis of cutaneous mast cell tumors and to lymph nodes for the diagnosis of lymphoma. One has the option of subsequently performing the suction technique if the initial specimen is nondiagnostic. This technique has the advantage of minimizing blood contamination and possibly reducing the risk of cell breakage.

To expel the specimen from the needle with either technique, one may need to remove the needle from the syringe, aspirate 1 to 2 mL of air into the syringe, replace the needle (while holding the tip of the needle over the glass slide), and gently express the specimen. Multiple slide preparations should be made when possible. Scrapings of cutaneous lesions can be used for sample collection. Ulcerated lesions often have superficial bacterial contamination, necessitating aggressive scraping of the deeper aspect of the lesion to obtain a reasonably diagnostic specimen.

The risks associated with fine-needle aspiration biopsy include rupture of an encapsulated inflammatory process, dissemination of an infectious agent, “seeding” of neoplastic cells in the needle tract, and hemorrhage. Each risk factor must be weighed against the benefits of diagnosis and the impact on the long-term outcome of the disease process. It is probable that neoplastic cells are displaced into unaffected tissue with relative frequency during fine-needle aspiration biopsy. Clinical studies indicate that neoplastic implants are uncommon, probably as a consequence of cytotoxic mechanisms associated with the immune system and other related inflammatory responses. However, despite the low risk, it is prudent to locate the needle track in the field of anticipated surgery so that it is removed along with the lesion. Similarly, when a suspected infectious process that involves an internal organ is being investigated, surgical readiness is advisable.

The microscopic examination of a fluid specimen is an important test in differential diagnosis of effusions. All specimens should be placed in an ethylenediaminetetraacetic acid (EDTA) tube to prevent possible clot formation. Since cellularity varies, both direct and sedimentation preparations should be routinely made. A conical plastic tube is ideally suited for obtaining a “cell pellet” by centrifugation. The same recommendation is made for specimens obtained by bronchoalveolar washing or lavage and for urine specimens. Cytocentrifuges that provide concentrated cell preparations of fluids that are low in cellularity are available. They are cost-effective in support of high-volume use and are highly recommended for making slide preparations from cerebrospinal fluid. Several direct slide preparations should always accompany fluids that are submitted to an outside laboratory in case the question of artifact arises. When slides are being directly prepared from a fluid

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specimen, *all* the fluid used for making the preparation should remain on the slide. The excess fluid may contain diagnostic clumps of cells that are discarded if they are dragged off the end of the slide; this is referred to as *the edge-of-the-cliff phenomenon*. The spreader (pusher) slide should be stopped approximately 1 cm ( $\frac{1}{2}$  inch) from the end of the specimen slide, and the excess fluid should be allowed to flow slightly backward (toward the start point) and allowed to dry. This technique also serves to concentrate cells in hypocellular fluids. A small hair dryer mounted on a stand facilitates the drying process, especially in geographic locations with high humidity.

Cytologic slide preparations of fluids that have a very low protein concentration (e.g., transudates, cerebrospinal fluid, urine) are problematic because the cells tend to be washed off during the staining process. Special slides can be prepared for these types of cytologic specimens. Serum, harvested from a clotted blood specimen, is used to thinly coat a number of glass slides. Once dry, these slides can be used immediately or be labeled as *serum-coated* and placed in an appropriate slide-holding container (a plastic slide holder with a snapping lid is ideal) and stored in the freezer until they are needed. After their removal from the freezer, the slides must be completely dry before the cytologic specimen is applied. If not, the hypotonic water condensation will lyse the cells. Because serum is a great medium for bacterial growth, one of the stored slides should be occasionally stained and examined if the slides are not used for several months.

## 17.2.2 Staining the Specimen (Box 16-1)

The staining process also requires a certain degree of “art and practice.” One of the most common problems encountered in cytology is an understained specimen. This is especially true for the thicker preparations such as aspirates from lymph nodes, liver, and bone marrow. Factors involved in understained specimens include inadequate exposure to the staining solutions, dilution and weakening of the solutions over time, and cytologic preparations that are simply too thick. The length of time for “dipping” in each of the solutions depends on the age of the stain and the cellularity (thickness) of the sample. The active process of “dipping” ensures exposure of the specimen to stain. Short times are adequate for hypocellular samples that have a low protein content such as transudates, whereas longer times are required for thick specimens. Adequate fixation of thick specimens before staining is important; specimens cannot be overfixed. After staining, the preparation is washed with cold running tap water for at least 15 seconds to remove excess stain and debris. The slide is air-dried and examined with the 20× objective. Understained specimens can be placed in either stain solution 1 or solution 2 again. An overstained specimen can be tempered by dipping it in the fixative several times, although the staining quality sometimes appears to be altered. Some quick stains have a tendency to overstain nuclear chromatin or accentuate nucleoli compared with Wright and Wright-Giemsa stains. Descriptive cytologic features may be obscured in lymphoid and bone marrow specimens.

### 17.2.2.1 BOX 16-1 Suggested Procedure for Staining Cytologic Specimens with Diff-Quik Solutions

Fixative: 60 to 120 seconds

Solution 1: 30 to 60 seconds

Solution 2: 5 to 60 seconds\*

Rinse under cold tap water: 15 seconds

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Examine staining adequacy by using low power; eosinophilia or basophilia can be enhanced by returning to solution 1 or solution 2, respectively, followed by a rinse.

Air-dry and examine.

Modified from Henry MJ, Burton LG, Stanley MW, Horwitz CA: Application of a modified Diff-Quik stain to fine needle aspiration smears: rapid staining with improved cytologic detail. *Acta Cytol* 1987;31:954-955.

Suggested times are based on fresh stains; with time and use, the stains weaken and longer times will be required. Consistently understained specimens are an indication for replenishing with fresh stain.

\* The shortest times are suggested for hypocellular fluids that are low in protein such as transudates, cerebral spinal fluid, and urine sediments.

## 17.2.3 Examination of the Cytologic Specimen

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The microscopic examination of the cytologic specimen is similar to that used for assessment of a blood smear.

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The entire specimen is scanned by using a low-power objective (10× or 20×); and sample adequacy, stain quality, and unusual findings such as cell clumps or structures are noted. Emphasis is given to the “feathered” end of the slides made from fluid specimens. Once an overall impression of the specimen is attained, high power (40× with a coverslip or 50× oil) is used to define the cell structure. Microscopic findings should be routinely and immediately recorded; it is important to put initial impressions on paper. These initial findings can be expanded or tempered during the detailed examination of the specimen. There are several basic statements that should be addressed for each specimen examined. Every cytologic description is initiated with the statement: “The predominant cell type is ....” Making a commitment to the definition of cell type is a critical first step in defining the cytologic process. If definition of the predominant cell type is problematic, a decision may be made to seek assistance, with the knowledge that the specimen is of adequate quality.

## 17.2.4 Sample Shipment and Identification

Plastic or Styrofoam containers prevent slide breakage during shipment or mailing of cytologic specimens. Flat cardboard or plastic slide containers are acceptable for courier handling but may not offer adequate protection in the mail. Cytologic slide preparations should never be placed in the same shipping container as formalin-fixed tissues. Making a cytologic preparation in the same room as an open formalin container can cause cell damage sufficient to violate the diagnostic integrity of the specimen. Exposure to formalin fumes causes nucleated cells to stain a bluish color with indistinct cellular detail. Erythrocytes may appear green or greenish blue.

Glass slides with a “frosted” end should be used for all cytologic preparations. The name(s) of the owner(s), patient/case number, and date are written on the frosted section with a pencil, never a felt-tipped pen. The signalment, the aspiration site, and history associated with the lesion should be included on the submission form. It is rare to hear a pathologist complain that too much information is provided with specimens. Once the reported findings are reviewed by the clinician, a subsequent phone conversation with the pathologist that provides missing or new information can sometimes facilitate a more conclusive interpretation, if necessary.

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## 17.3 CLASSIFICATION (Box 16-2)

### 17.3.1 Anticipate the Expected, Expect the Unexpected

When a known tissue is aspirated, whether the specimen is compatible with the expected tissue should be determined; that is, one should anticipate the expected. Defining the predominant cell type often accomplishes this objective. Making observations that support or deny what is cytologically expected is critical to proceeding in the interpretive process. For example, if the tissue aspirate is thought to be from a lymph node and the specimen is compatible with lymphoid tissue, then the subsequent observations are used to classify the lymph node cytologically. However, if the tissue is not compatible with the anticipated observation of lymphoid constituents, then the specimen remains an unknown and requires additional cytologic definition. A frequent representative situation is the inadvertent aspiration of the salivary gland instead of the submandibular or retropharyngeal lymph node.

Classifying a cytologic specimen initially into general categories may be adequate for diagnosis or provide sufficient findings to guide further cytologic interrogation. General categories that may be used based on the principal findings are inflammation versus noninflammation and neoplastic versus nonneoplastic. Algorithms are available that provide further subclassification and guidance for advanced diagnostic classification of selective specimens.

#### 17.3.1.1 BOX 16-2 Indications for the Use of Cytology

Effusions—thoracic and abdominal

Urine sediments, urinary bladder washing

Vitreous/aqueous infiltrates

Prostate—direct aspirate, washing

Pulmonary/nasal lesion—direct aspirate, bronchoalveolar/nasal washing/lavage

Lymphadenopathy—focal, generalized

Cutaneous/subcutaneous mass, ulcerative lesion

Diffuse organomegaly—liver, spleen, kidney

Unidentified abdominal mass

Evaluation of a mass or lesion discovered intraoperatively

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### 17.3.2 Inflammation versus Noninflammation

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The segmented neutrophil is an easily recognized cell marker of suppuration, either sterile or septic. The predominance of neutrophilic inflammation suggests acute disease and/or an infectious or irritative pathogenesis, since the attendant chemotactic factors attract the circulating neutrophils to rapidly migrate to the affected tissue. Bacteria, gram-positive or gram-negative, generally stain a deep blue-purple with Romanowsky

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stains. A Gram stain is used to determine whether the organism is gram-positive or gram-negative. The observation of neutrophils that lack nuclear integrity (e.g., those with mushy nuclear chromatin—karyolysis—caused by the release of digestive lysozymes) is a hint that bacteria may be present. The 50× or 100× oil objective is used to confirm the presence of bacteria (Plate 20). Their visual identification is especially important in specimens (e.g., synovial fluid and effusions) because the specimen may culture negatively. This can occur because of too few organisms, lysosomal degradation of the organisms, or the presence of antibiotics in the specimen before or during antibacterial treatment. Macrophages and lymphocytes, along with lesser numbers of neutrophils, generally constitute the classification of chronic inflammation (Plate 21). At times, macrophages may morphologically mimic neoplastic cells.

Tissue cells, both mesenchymal and epithelial, can undergo hyperplasia or hypertrophy as a reaction to inflammation. The cells may morphologically mimic carcinoma or sarcoma cancer cells. The predominance of the inflammatory cells compared with suspected neoplastic cells tempers the conclusion of malignancy. Tissues such as the nasal cavity, lung, urinary bladder, prostate, and mesothelium are notorious for undergoing robust hyperplasia in response to inflammation.

## 17.3.3 Neoplasia versus Nonneoplastic Disease

### 17.3.3.1 Round Cell (Discrete Cell) and Distinctive Cell Tumors

The noninflammatory cytologic specimen may represent either neoplasia or nonneoplastic tissue (Plates 22 and 23). When a neoplastic cell population is considered, the first goal in cytology is to place the predominant cell type into a general classification. The distinctive morphologic features of round cell (discrete cell) tumors (e.g., lymphomas, mast cell tumors, plasma cell tumors, transmissible venereal cell tumors, histiocytomas, and distinctive cell tumors), melanocyte tumors, and lipomas tend to be easily recognized (Plates 24 to 25262829).

### 17.3.3.2 Epithelial and Mesenchymal Tumors Box 16-3)

Epithelial malignancies (carcinomas) and mesenchymal malignancies (sarcomas) are more difficult to recognize cytologically than round cell and distinctive cell tumors. A specific morphologic diagnosis is often unlikely. The goal is to differentiate malignancy and benignity. The more abnormal morphologic features and altered staining properties that are present, the greater is the likelihood of malignancy. Epithelial cells are generally oval to polygonal and tend to form variably sized sheets and clumps (Plate 30). The cells are generally linked to one another by membrane structures referred to as *cell-to-cell cohesion*. The mesenchymal classification includes connective tissue, skeletal tissue, and blood and lymphatic vessels. The cell types are stellate to spindle to ovoid in shape (Plate 31). Reddish pink irregular ribbons of intercellular matrix may be observed swirled among the cells. Histologic examination to assess architecture is required when the cytologic determination is inconclusive or when a specific morphologic diagnosis must be made.

### 17.3.3.3 Neuroendocrine Tumors

Malignant tumors of neuroendocrine origin are deceptive, since they can appear morphologically similar to round cell tumors and often do not demonstrate morphologic variation. Thyroid, adrenal, and pancreatic tumors show similar morphologic characteristics; they are composed of a relatively uniform cell population, often containing free nuclei from ruptured cells because of their fragility. The cells often stain uniformly and

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appear to lack well-delineated cell borders. The apocrine gland adenocarcinoma of the anal sac has a similar nonaggressive appearance, even when it is found as a metastasis to the sublumbar lymph node.

## 17.3.3.3.1 BOX 16-3 Morphologic Variables of Epithelial and Mesenchymal Neoplasms Suggestive of Malignancy

Variable cell sizes (anisocytosis)

Variable cell forms (pleomorphism)

Variable cytoplasmic staining intensity

Variable nuclear sizes (anisokaryosis)

Variable nucleolar sizes and shapes (may be multiple)

Variable nuclear to cytoplasm ratio

## 17.3.4 Effusions

Cytologic findings in body cavity fluids can often provide a classification of inflammation, noninflammation, or neoplasia. For noninflammatory specimens, the measurement of the total protein with a refractometer differentiates a transudate from a modified transudate. The pathophysiologic process responsible for their formation may be suggested by those findings when combined with the other clinicopathologic findings. The presence of mesothelial cells adds a potential element of distraction. The cell is a morphologic chameleon that can take on a variety of appearances, including immature basophilic clumps of epithelial cells with a carcinoma-like look, pale-staining vacuolated epithelial cells compatible with a macrophage look, and large immature basophilic lymphoblast-like cells.

## 17.3.5 Lymphoid Tissue

Cytology is commonly used for the evaluation of lymphadenopathy to differentiate between reactive lymphoid hyperplasia and lymphoma. Reactive lymphoid hyperplasia is cytologically defined by the prominence of a relatively uniform population of small lymphocytes (>50%) along with increased numbers of variably sized lymphocytes that include medium, large, and blastic forms (Plate 23). Observation of a relatively uniform population of predominantly (>50%) medium, large, or blastic lymphocytes supports a cytologic diagnosis of lymphoma (Plate 24). One approach to determine the approximate percentage of a cell type is to use an oil objective, count two to three fields of 100 total lymphocytes, and note how many are small lymphocytes. Lymphocyte size determination is aided by the use of other cell types that may be coincidentally present. The small lymphocyte with its barely visible scant rim of cytoplasm approximates the size of an erythrocyte and is smaller than a neutrophil. When this criterion is used as an example, a diagnosis of lymphoma is made if the majority of the lymphocytes in an aspirate from an enlarged lymph node are relatively monomorphic and larger than a neutrophil. A similar cytologic strategy can be used to define lymphoma in aspirates from other organs such as the liver, spleen, and kidneys. An additional advantage for the identification of lymphoma in these organs is that large numbers of lymphocytes are not expected findings.

In dogs, lymphoma is a relatively common differential consideration for generalized lymphadenopathy; however, a caveat must be realized for cats. A syndrome of generalized lymphadenopathy, referred to as

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*distinctive lymph node hyperplasia*, develops in young adult cats with or without other clinical signs. Cytologic examination can reveal a marked polymorphic lymphoid picture in which small lymphocytes represent the minority and huge lymphoblasts are observed. Noteworthy is that erythrophagocytosis can usually be detected by adequate scanning. There can be sufficient distortion of the architecture that even the histologic examination of the lymph node can be suggestive of lymphoma. The majority of cases spontaneously resolve weeks to months later without subsequent development of neoplasia.

Specimens from peripheral and internal lymph nodes can be examined for metastatic disease when indicated. A low-power scan of the smear made from a suspected metastatic lymph node may permit detection of clumps of cells that differ from lymphocytes.

## 17.3.6 Cutaneous and Subcutaneous Lesions

Cutaneous and subcutaneous lesions are cytologically challenging because of the histologic complexity of skin and the wide range of potential diseases that can involve the skin. A cytologic specimen may provide a specific diagnosis (e.g., mast cell tumor) or a general classification (e.g., inflammation) or may lack exfoliated cells. A biopsy is necessary when cytology provides inadequate findings for differential diagnosis.

## 17.3.7 Liver

Hepatomegaly is the primary indication for fine-needle aspiration biopsy of the liver. Normal hepatocytes are large polygonal cells, often found in cohesive clumps. The round nuclei are surrounded by abundant lightly basophilic cytoplasm that may contain specks of dark-staining bile pigment. Sheets of biliary epithelium may be encountered. These tightly clumped cuboidal cells are smaller than hepatocytes with central round nuclei and scant basophilic cytoplasm. An abundance of hepatocellular dark pigment is indicative of cholestasis. Biliary casts may be observed outlining the canalicular space on the surface of the hepatocytes. Hepatocellular vacuolation occurs as a result of lipid accumulation and is dramatic in cats with hepatic lipidosis. These distinctly round structures with clear lumens range from multiple small to a single large form that pushes the nucleus to the periphery of the cell. Lesser degrees of hepatocellular accumulation of lipid are common sequelae of both toxic and metabolic disorders that involve the liver. Excessive corticosteroids cause moderate to marked glycogen accumulation in the canine liver. On microscopic examination, the affected hepatocytes are larger and rarified cytoplasm, that is, wispy cytoplasmic swirls. The nuclei usually maintain a central location. Carcinomas, sarcomas, and round cell tumors can be identified in liver specimens obtained by fine-needle aspiration biopsy. Histologic examination of a liver biopsy specimen is the best procedure for identifying and characterizing inflammatory liver disease. Cytologic findings may be misleading and cannot be used to describe hepatic architecture.

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## 17.3.8 Spleen

Splenomegaly is the primary indication for fine-needle aspiration biopsy of the spleen. The aspirate from a nondiseased spleen often contains peripheral blood cells. Occasionally, a few monomorphic spindle cells, representing fibrocytes or endothelial cells, are seen. The predominant nucleated cell population is lymphoid, and assessment of lymphoid cells is done by using the criteria established for the lymph node. Small lymphocytes predominate with a lesser population of polymorphocytes and lymphoblasts. Occasional plasma cells, macrophages, and mast cells can be seen. Increased numbers of polymorphocytes, lymphoblasts, and plasma cells are seen in reactive spleens, especially in association with gastrointestinal inflammation. A marked plasmacytosis can be observed in canine ehrlichiosis and feline immunodeficiency infections.

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Neoplasms composed of lymphocytes, plasma cells, mast cells, and myeloid cells readily exfoliate. Lymphoma is characterized by a monomorphic population of lymphoid cells; the criteria are discussed in the section on lymph nodes. Extraskelatal plasma cell tumors can occur in both the spleen and the liver. Splenic mast cell tumors are easily identified. Myeloproliferative disease involving any of the cell lines can involve the spleen. Generally, it is characterized by a relatively monomorphic population of predominantly immature myeloid elements. Concurrent evaluation of peripheral blood and bone marrow is necessary to fully characterize the neoplastic process. The use of ultrasonography for the identification of focal, cavitational lesions is valuable for the characterization of hemangiosarcomas, which are the most common splenic neoplasias in dogs. Splenic hemangiosarcoma is difficult to diagnose by means of aspiration cytology. The specimens are often composed of peripheral blood elements. Examination of a buffy coat preparation from such a specimen can enhance the probability of finding atypical mesenchymal cells that have spontaneously exfoliated into a blood-filled cavity of the neoplasm. The cells will have a spindle to elliptical shape with indistinct cytoplasmic borders and wispy “tails” and demonstrate the criteria for malignancy. The risk of the fragile neoplastic tissue leaking and “seeding” the abdomen or blatantly rupturing is high, and the procedure is discouraged.

## 17.3.9 Gastrointestinal Tract

Gastrointestinal specimens may be obtained by endoscopic sampling or from touch imprints of surgical biopsy specimens. The expected cell type is a monomorphic cuboidal to columnar epithelial cell. The presence of other cell types can be suggestive of either inflammation or neoplasia. The predominance of a lymphocytic plasma cell mixture or eosinophils is indicative of their respective disease syndromes. Fungi that cause histoplasmosis, candidiasis, and zygomycosis (phycomycosis) and algae that cause protothecosis can be readily identified. Cytology is useful in detecting spiral organisms in gastric samples. Lymphoma and mast cell tumors exfoliate readily.

## 17.3.10 Prostate

Prostatomegaly and/or the finding of hematuria and pyuria in an intact, older male dog are indications for cytologic investigation of the prostate. A urethral discharge that is not preputial in origin is commonly associated with prostatic disease. After the prepuce has been cleansed, the discharge can be collected and examined. Prostatic fluid is sterile, and finding a uniform population of bacteria in a suppurative surrounding is indicative of prostatitis. Collection of an ejaculate is another approach for obtaining a cytologic specimen, since more than 95% of it is contributed by the prostate. Collection of the latter part of the ejaculate provides the more diagnostically useful specimen for culture and microscopic examination. Prostatic massage is another technique used to capture a cytologic specimen. It aids in differentiating disease of the urinary bladder from prostatic disease. Fine-needle aspiration biopsy of the prostate is conducted with either a perirectal or transabdominal approach. Aspiration of a suspected abscess (when the suspicion is based on clinical, laboratory, and ultrasonographic findings) is not recommended.

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## 17.3.11 Kidneys

Renomegaly is the primary indication for fine-needle aspiration biopsy of the kidneys. Ultrasound-guided fine-needle aspiration biopsy permits the cytologic investigation of a focal lesion. The kidney can be restrained against the abdominal wall by digital pressure in cats and small dogs to facilitate the aspiration procedure. The kidney is a fibrous organ that spontaneously yields few of its elements. Segments of tubules and epithelial and small spindle-shaped cells can be aspirated along with peripheral blood components from a nondiseased kidney.

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Epithelial cells from the feline kidney often contain multiple, punctate vacuoles representing areas of lipid that dissolved during the staining process. Carcinomas and lymphomas are common tumors of the kidney.

## 17.3.12 Urinary Bladder

The preparation of cytologic specimens of urine is covered in an earlier section. Plump transitional epithelial cells with abundant relatively clear cytoplasm line the urinary bladder and urethra. They can demonstrate remarkable morphologic changes when hyperplasia is stimulated by inflammation. Consequently, the criteria of malignancy must be applied cautiously when inflammation is present concurrently. If one is in doubt, medical management of the bacterial cystitis, followed by a second cytologic evaluation, is a reasonable option. The transitional cell carcinoma is the most common tumor of the urinary bladder in dogs and cats. It is identified by using the criteria of malignancy.

## 17.3.13 Lungs

Evaluation of the respiratory tree by transtracheal wash or bronchoalveolar lavage is used to evaluate airway-related diseases such as bronchopneumonia and allergies. Identification of neoplastic, mycotic, or protozoal diseases by examination of airway material is possible only if cells or organisms are exfoliated into the airway. Obtaining an adequate sample is crucial when these techniques are used. Respiratory epithelial cells are observed in these samples. These cells are columnar to cuboidal and often ciliated. Oval nuclei with granular chromatin are situated toward the base of the cell, and the cytoplasm is lightly basophilic. Goblet cells may contain eye-catching pink to mauve granules. Mucus is often present in samples from the respiratory tract as ribbons of eosinophilic material. Occasionally, this material is found in tightly coiled forms referred to as *Curschmann's spirals*. They apparently represent mucus casts of the lower airway.

Fine-needle aspiration biopsy of the lung parenchyma is rewarding when the interstitial disease is diffuse or when large focal lesions are identified radiographically. Both infectious and neoplastic diseases can be identified by means of cytologic examination. The lung is a primary site of involvement in both systemic histiocytosis of Bernese mountain dogs and canine malignant histiocytosis. Systemic histiocytosis is a histiocytic proliferative disorder confined to Bernese mountain dogs. Malignant histiocytosis has been identified in several breeds of dogs, with a suggested predisposition in Bernese mountain dogs, golden retrievers, and flat-coated retrievers. Malignant histiocytosis can be cytologically difficult to differentiate from granulomatous inflammation, large cell anaplastic carcinoma, histiocytic lymphoma, and pulmonary lymphomatoid granulomatosis.

## 17.4 ADDITIONAL READING

PH Brown, CD Hopper, DA Harbour: Pathological features of lymphoid tissues in cats with natural feline immunodeficiency virus infection. *J Comp Pathol.* **104**, 1991, 345–355.

258

M Caniatti, P Roccabianca, E Scanziani, et al.: Canine lymphoma: Immunohistochemical analysis of fine-needle aspiration biopsy. *Vet Pathol.* **33**, 1996, 204–212.

259

RF Carter, Valli, VEO: Advances in the cytologic diagnosis of canine lymphoma. *Semin Vet Med Surg.* **3**, 1988, 167–175.

RL Cowell, RD Tyler: In *Diagnostic cytology of the dog and cat*. 1989, American Veterinary Publishers, Goleta, Calif, 1–259, New references added to the third edition.

# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

- \*G De Vico, A Sfacteria, P Maiolino, et al.: Comparison of nuclear morphologic parameters in cytologic smears and histologic sections of spontaneous canine tumors. *Vet Clin Pathol.* **31**, 2002, 16–18.
- RW Else, JW Simpson: Diagnostic value of exfoliative cytology of body fluids in dogs and cats. *Vet Rec.* **123**, 1988, 70–76.
- GL Griffiths, JH Lumsden, Valli, VEO: Fine needle aspiration cytology and histologic correlation in canine tumors. *Vet Clin Pathol.* **13**, 1984, 13–17.
- R Leveille, BP Partington, DS Biller, et al.: Complications after ultrasound-guided biopsy of abdominal structures in dogs and cats: 246 cases (1984-1991). *J Am Vet Med Assoc.* **203**, 1993, 413–415.
- T Livraghi, B Damascelli, C Lombardi, et al.: Risk in fine-needle abdominal biopsy. *J Clin Ultrasound.* **11**, 1983, 77–81.
- S Mair, F Dunbar, PJ Becker, et al.: Fine needle cytology—is aspiration suction necessary? A study of 100 masses in various sites. *Acta Cytol.* **33**, 1989, 809–813.
- DJ Meyer: The management of the cytology specimen. *Comp Contin Educ Pract Vet.* **9**, 1987, 9–16.
- DJ Meyer, P Franks: Effusion: Classification and cytologic examination. *Comp Contin Educ Pract Vet.* **9**, 1987, 123–128.
- SC Mooney, AK Patnaik, AA Hayes, et al.: Generalized lymphadenopathy resembling lymphoma in cats: Six cases (1972-1976). *J Am Vet Med Assoc.* **190**, 1987, 897–900.
- FM Moore, WE Emerson, SM Cotter, et al.: Distinctive peripheral lymph node hyperplasia of young cats. *Vet Pathol.* **23**, 1986, 386–391.
- DA O'Keefe, CG Couto: Fine-needle aspiration of the spleen as an aid in the diagnosis of splenomegaly. *J Vet Intern Med.* **1**, 1987, 102–109.
- V Perman, RD Alsaker, RC Riis: In *Cytology of the dog and cat*. 1979, American Animal Hospital Association, Denver, 1–159.
- \*RE Raskin, DJ Meyer: In *Atlas of canine and feline cytology*. 2001, WB Saunders, Philadelphia.
- BA Rideout, LJ Lowenstine, CA Hutson, et al.: Characterization of morphologic changes and lymphocyte subset distribution in lymph nodes from cats with naturally acquired feline immunodeficiency virus infection. *Vet Pathol.* **29**, 1992, 391–399.
- Ross DL, Neely AE: Textbook of urinalysis and body fluids. Norwalk, Conn, 1983, pp 1-336.
- RM Simpson, DJ Meuten: Development of a teaching laboratory aid for instruction of fine needle aspiration biopsy cytology technique. *Vet Clin Pathol.* **21**, 1992, 40–44.
- EH Smith: The hazards of fine-needle aspiration biopsy. *Ultrasound Med Biol.* **10**, 1984, 629–634.
- RF Smith: In *Microscopy and photomicroscopy: A working manual*. 1990, CRC Press, Boca Raton, Fla, 1–135.
- WL Spangler, MR Culbertson: Prevalence and type of splenic diseases in cats: 455 cases (1985-1991). *J Am Vet Med Assoc.* **201**, 1992, 773–776.
- WL Spangler, MR Culbertson: Prevalence, type, and importance of splenic diseases in dogs: 1,480 cases (1985-1989). *J Am Vet Med Assoc.* **200**, 1992, 829–834.
- M Takeda: In *Atlas of diagnostic gastrointestinal cytology*. 1983, Igaku-Shoin Medical Publishers, New York, 229.

## Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

---

JH Vos, van den Ingh TSGAM, FN van Mil: Non-exfoliative canine cytology: The value of fine needle aspiration and scrapping cytology. *Vet Q.* **11**, 1989, 222–231.

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## <sup>1</sup> Chapter Color Plates

Plate 1 Regenerative anemia in a dog with increased polychromasia and anisocytosis. A metarubricyte is present in the upper left and a Howell-Jolly body is present in an erythrocyte in the upper right. (Wright-Giemsa stain.)

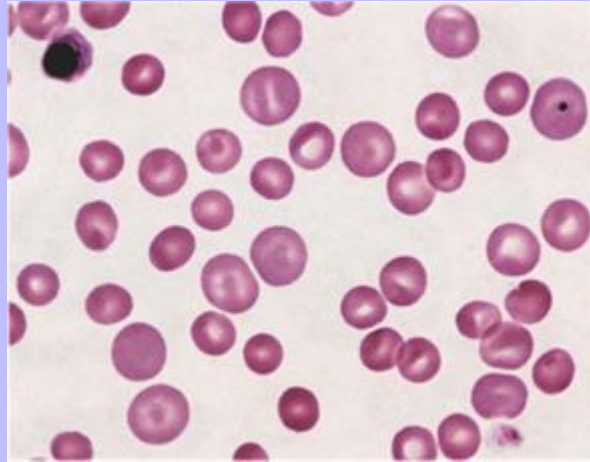
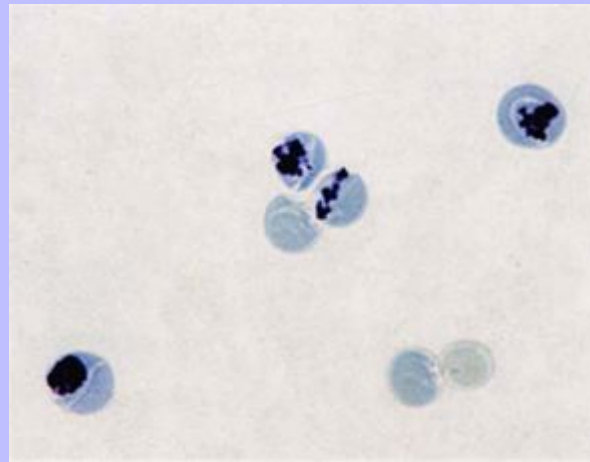


Plate 2 Four reticulocytes containing dark blue-staining material and three mature erythrocytes in a dog with a regenerative anemia. (Reticulocyte stain.)



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Plate 3 Hypochromic erythrocytes in blood from a dog with chronic iron deficiency anemia. (Wright-Giemsa stain.)

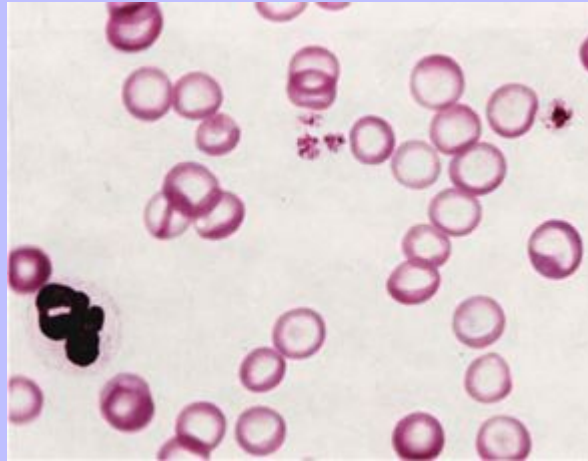
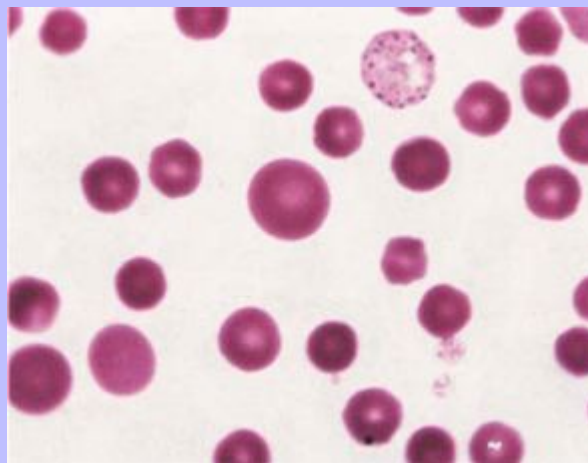


Plate 4 Regenerative anemia with increased anisocytosis in blood from a cow with anaplasmosis. A single *Anaplasma marginale* organism is present in an erythrocyte at the bottom and an erythrocyte with basophilic stippling is present in the top portion of the figure. (Wright-Giemsa stain.)



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Plate 5 Heinz body hemolytic anemia in a cat. Pale spots present within erythrocytes are poorly stained Heinz bodies. (Wright-Giemsa stain.)

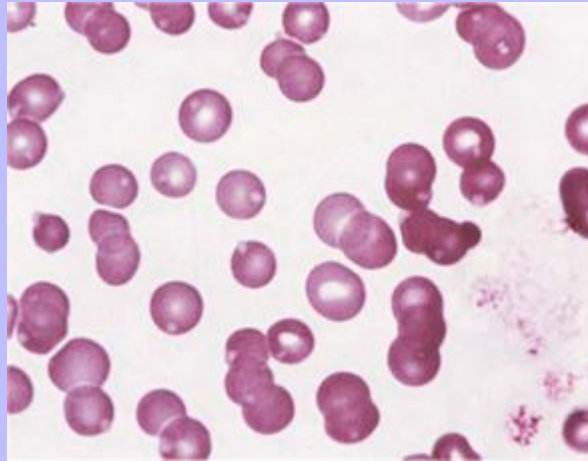
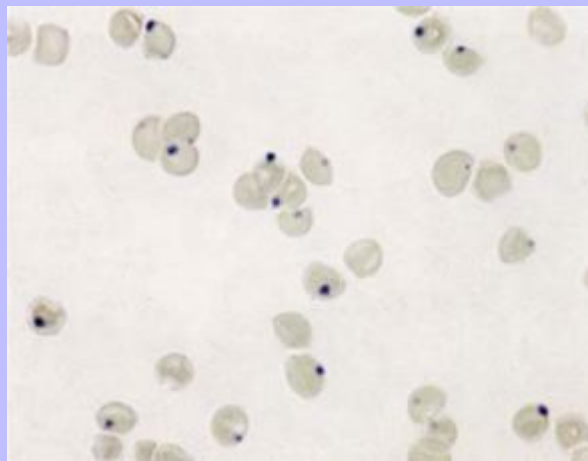


Plate 6 Heinz body hemolytic anemia in a cat. Blue-staining inclusions are Heinz bodies. (Reticulocyte stain.)



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Plate 7 Large numbers of *Mycoplasma haemosuis* organisms are present on and between erythrocytes in blood from a splenectomized pig. Most erythrocytes are echinocytes, an expected finding on porcine blood films. (Wright-Giemsa stain.)

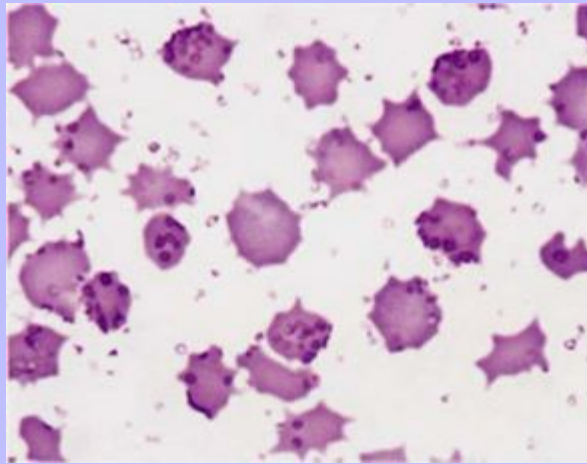
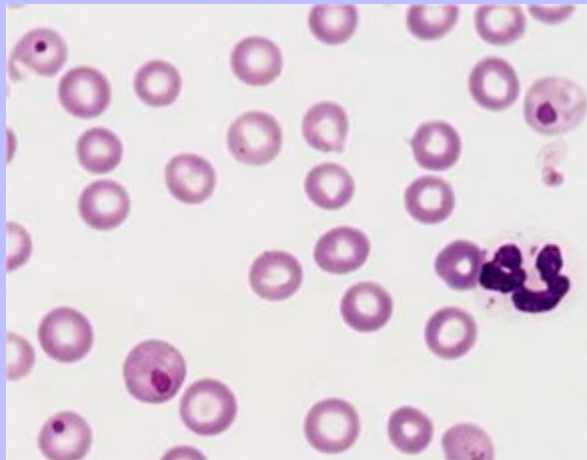


Plate 8 Blood from a dog with distemper. Red-staining distemper inclusions are present in a neutrophil and several erythrocytes. (Diff-Quik stain.)



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Plate 9 Eosinophilia in blood from a horse with an abdominal mast cell tumor.  
(Wright-Giemsa stain.)

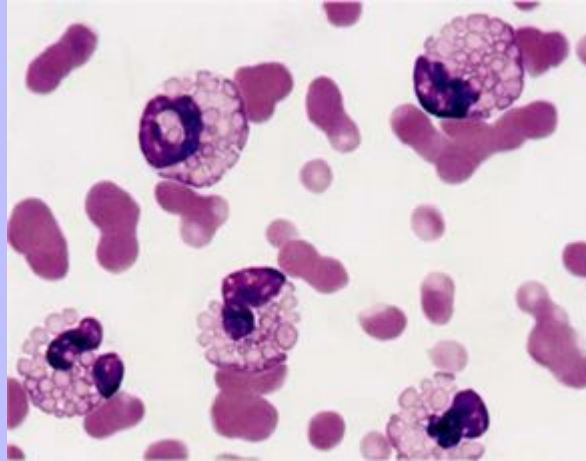
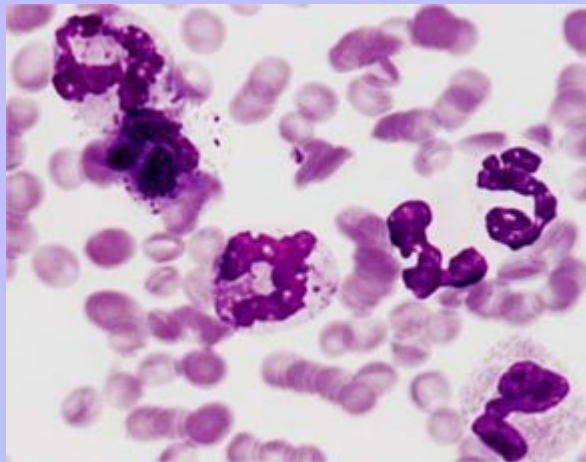


Plate 10 Three basophils (*center and left*), two neutrophils (*right*), and an eosinophil (*bottom right*) in blood from a cat with dirofilariasis. (Wright-Giemsa stain.)



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Plate 11 An eosinophil (*left*), basophil (*center*), and neutrophil (*right*) in blood from a dog. (Wright-Giemsa stain.)

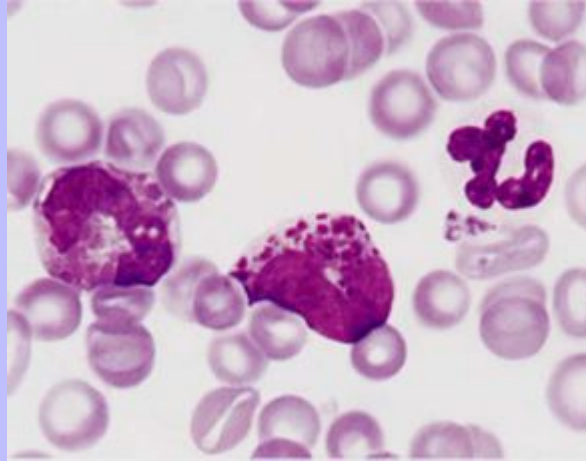
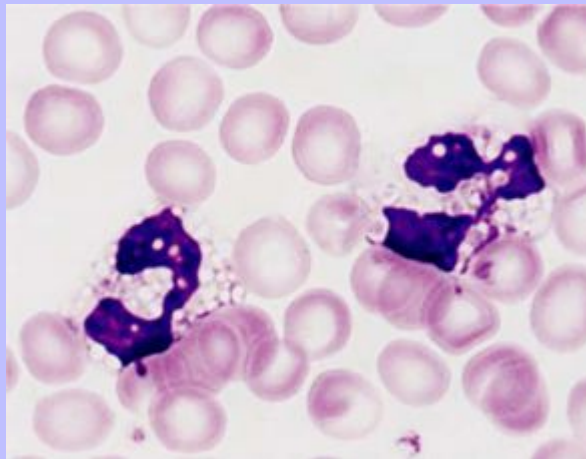


Plate 12 Two eosinophils with vacuolated cytoplasm in blood from a greyhound dog. (Wright-Giemsa stain.)



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Plate 13 A monocyte (left) and a reactive lymphocyte (*right*) in blood from a dog. (Wright-Giemsa stain.)

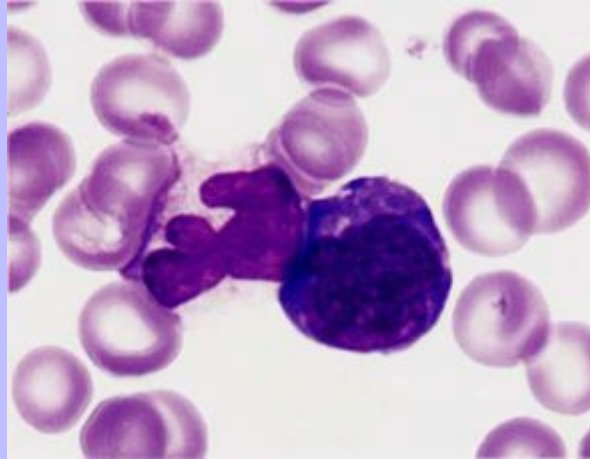
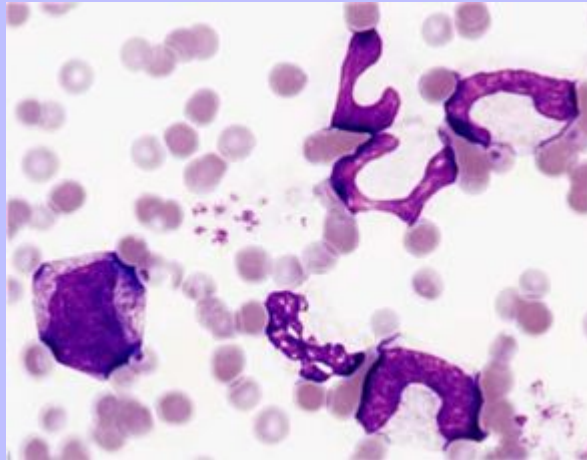


Plate 14 Toxic left shift in blood from a cat with a bacterial infection. A promyelocyte (*left*), four band neutrophils, and a mature neutrophil are present. The cytoplasm of the cells exhibits increased basophilia. (Wright-Giemsa stain.)



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Plate 15 A monocyte (*left*), neutrophil (*top*), and lymphocyte (*right*) in blood from a cow. (Wright-Giemsa stain.)

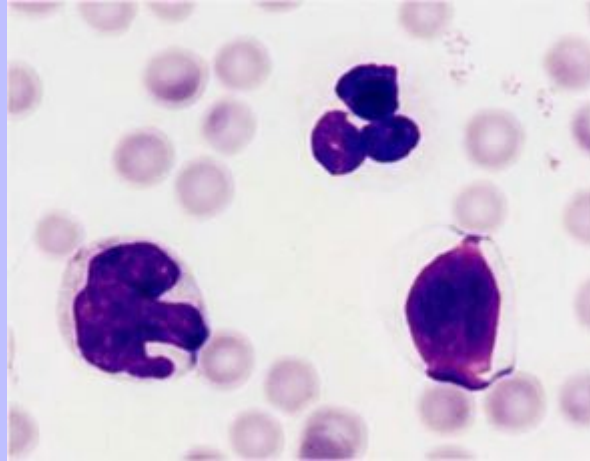
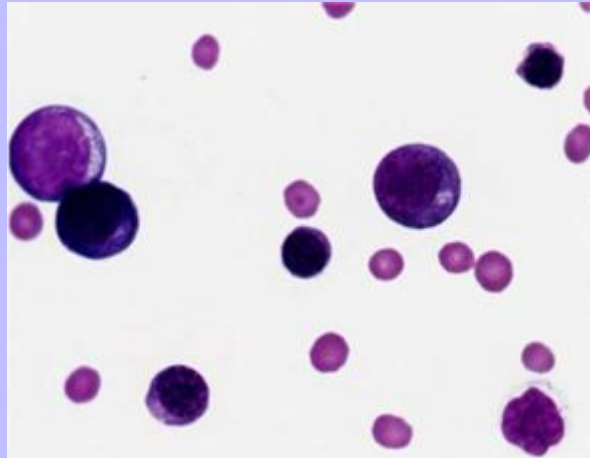


Plate 16 Blood from an anemic cat with an erythroleukemia (AML-M6). A myelocyte (*top left*), granular lymphocyte (*bottom right*), and five nucleated erythroid cells are present, but polychromasia is absent. (Wright-Giemsa stain.)



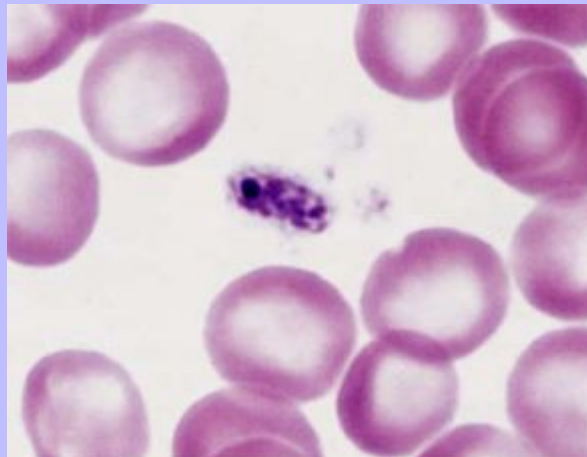
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Plate 17 Granular lymphocyte in blood from a dog. (Wright-Giemsa stain.)



Plate 18 *Anaplasma platys* morula in a platelet in blood from a dog. (Wright-Giemsa stain.)



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Plate 19 Maturation of canine erythroid and granulocytic cells as they appear in Wright-Giemsa-stained bone marrow aspirate smears. (Drawing by Dr. Perry Bain.)

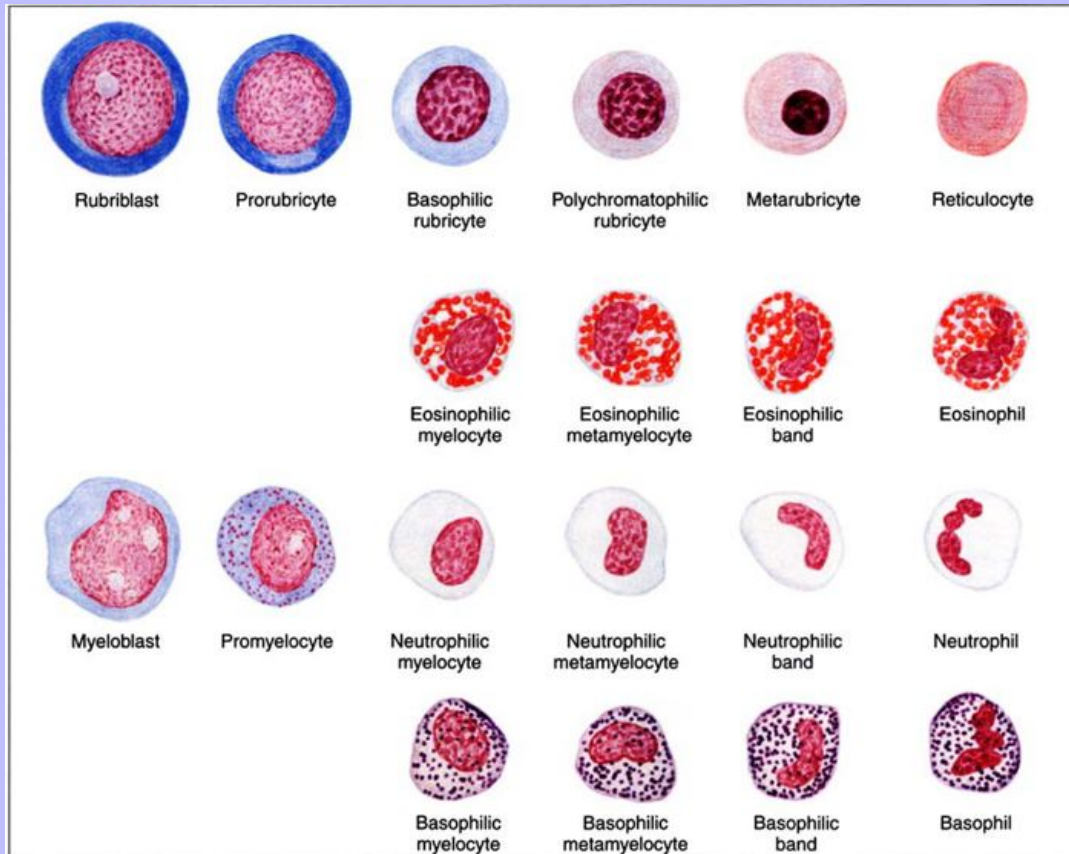
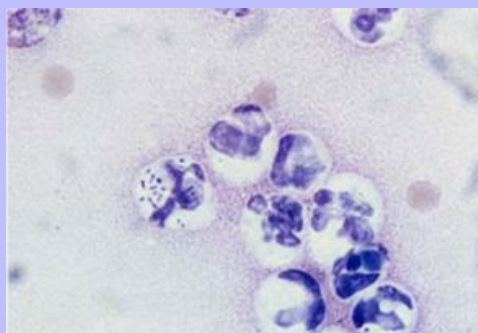


Plate 20 Septic inflammation—synovial fluid. A marked increase in neutrophils in the synovial fluid, some containing bacteria, characterizes this septic arthritis.



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Plate 21 Sterile inflammation—pleural fluid. Mixed inflammatory cells consisting of neutrophils and macrophages characterize this pleural fluid as a sterile exudate (total protein = 4.6 g/dL). Feline infectious peritonitis was later confirmed.

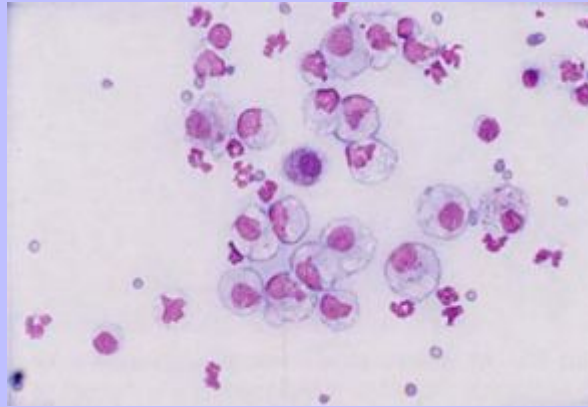
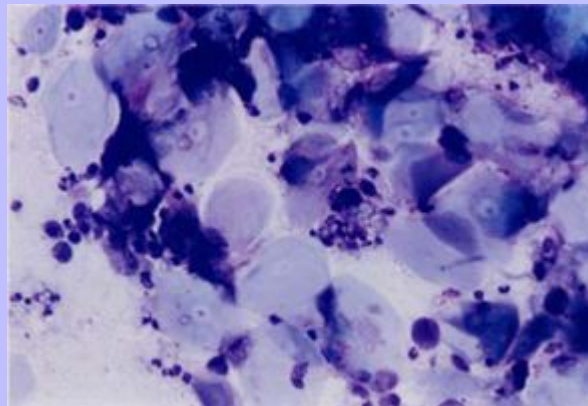


Plate 22 Noninflammatory, nonneoplastic cystic structure—skin mass. Abundant anuclear squamous cells and cellular debris characterize the skin mass as an epidermal inclusion cyst. Rupture of the cyst wall stimulates a mixed inflammatory reaction of neutrophils and macrophages (not shown).



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Plate 23 Noninflammatory, nonneoplastic hyperplasia—lymph node. The predominance of small lymphocytes with lesser numbers of a heterogeneous population of variably sized lymphocytes defines a hyperplastic (reactive) lymph node.

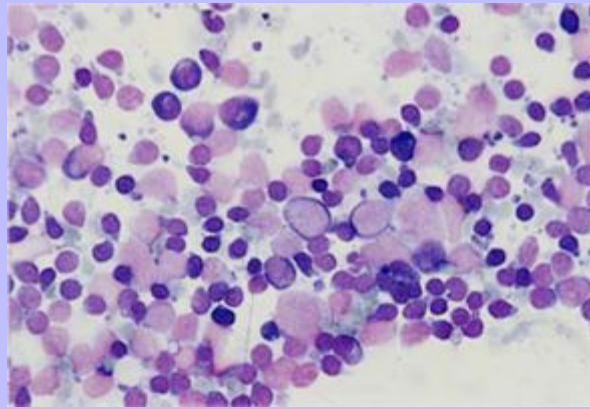
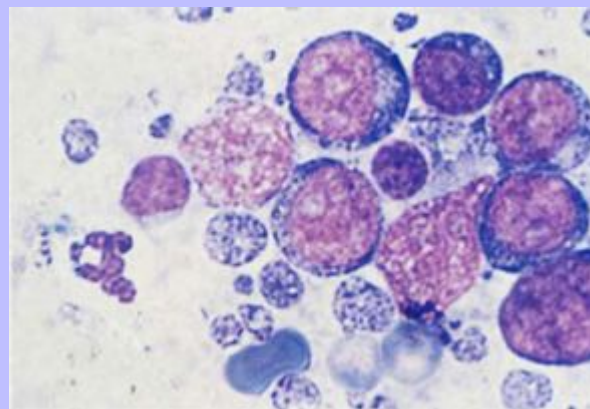


Plate 24 Noninflammatory, neoplastic, cytologically discrete cell—lymph node. The presence of primarily medium to large lymphocytes defines lymphoma. A small lymphocyte (*near center*) and a neutrophil (*far left*) are useful “micrometers” for the determination of the relatively large size of the cell population being evaluated. Small, variably sized, basophilic oval structures represent cytoplasmic fragments from ruptured fragile neoplastic cells and are referred to as lymphoglandular bodies; they have no diagnostic importance.



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Plate 25 Noninflammatory, neoplastic, cytologically discrete cell–skin mass. A mast cell tumor is characterized by a monotonous population of plump, discrete cells with abundant metachromatic granules in the cytoplasm that obscures the nucleus.

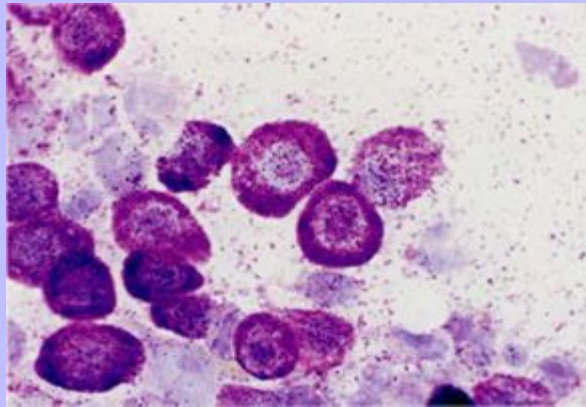
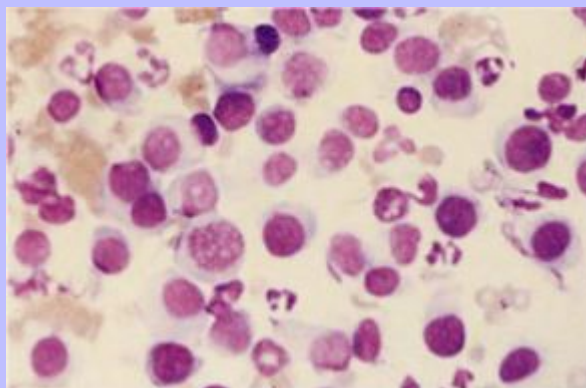


Plate 26 Noninflammatory, neoplastic, cytologically discrete cell–ulcerated skin mass. A histiocytoma is composed of discrete cells showing mild size variation with a round to slightly indented nucleus consisting of fine, lightly stained chromatin surrounded by variable amounts of slightly basophilic to clear cytoplasm. An admixture of erythrocytes, neutrophils, and small lymphocytes (*two dark oval cells at top center*) are useful cytologic “micrometers.” Small lymphocytes can become more numerous as the lesion undergoes spontaneous regression.



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Plate 27 Noninflammatory, neoplastic, cytologically discrete cell–skin mass. A plasmacytoma is composed of variably sized discrete cells with a round to oval nucleus consisting of darkstaining coarse chromatin surrounded by variable amounts of mildly to moderately basophilic cytoplasm. The morphology of an occasional cell closely resembles a normal plasma cell (*top, slightly left of center*). Multinucleated cells are often present as in this case. An admixture of erythrocytes (greenish-stained) and neutrophils are useful cytologic “micrometers.”

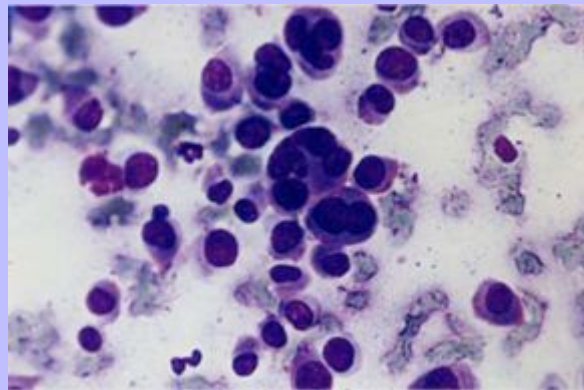
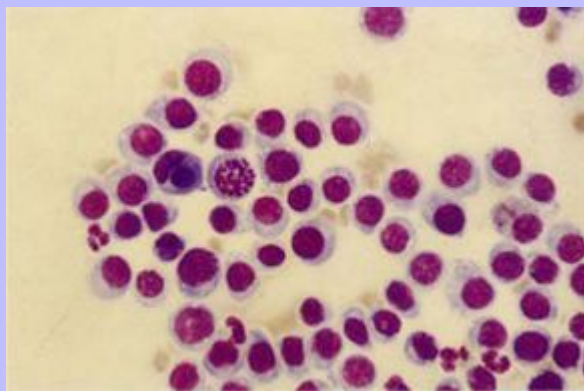


Plate 28 Noninflammatory, neoplastic, cytologically discrete cell–ulcerative preputial mass. A transmissible venereal cell tumor (TVT) is composed of variably sized discrete cells with a round to oval nucleus consisting of coarse chromatin with one or two notable nucleoli. The nucleus is surrounded by slightly basophilic cytoplasm that often contains variable numbers of punctate vacuoles. A mitotic figure is observed to the left of center. Erythrocytes and several neutrophils are useful cytologic “micrometers.”



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Plate 29 Noninflammatory, neoplastic, cytologically distinctive cell-oral mass. A melanoma is composed of aggregates of cells containing dense, dark granules (melanin) in the cytoplasm that occasionally obscure the nucleus. An occasional cell has clear cytoplasm with only sparse melanin pigment. When this poorly differentiated morphologic variant is the predominant cell type (amelanotic melanoma), the neoplasm can cytologically resemble an anaplastic carcinoma or anaplastic sarcoma.

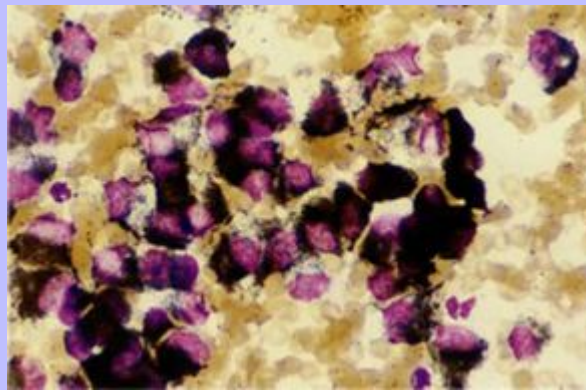
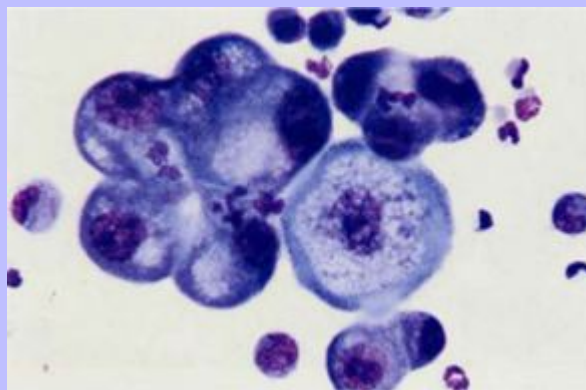


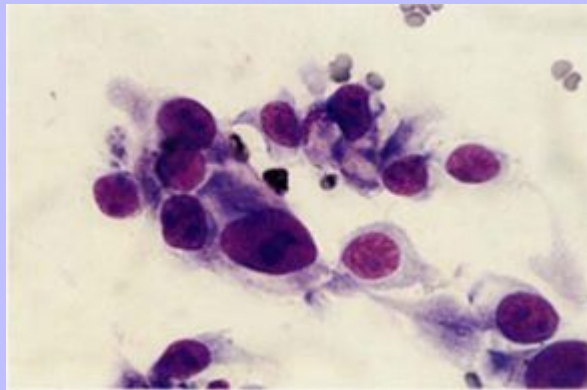
Plate 30 Noninflammatory, neoplastic-malignant epithelial neoplasm—pleural fluid. Single cell and small aggregates of huge oval cells that demonstrate moderate anisocytosis, anisokaryosis, and variable nucleus/cytoplasm ratio are the predominant cell type. The voluminous cytoplasm stains variably basophilic and is occasionally vacuolated. The cytologic findings are indicative of a carcinoma. The rosette-like formation resembles an attempt to form an acinus, suggesting further characterization as an adenocarcinoma. The surrounding neutrophils accentuate the gigantism of the neoplastic cells.



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Plate 31 Noninflammatory, neoplastic-malignant mesenchymal neoplasm—ulcerated, poorly circumscribed ulcerated skin mass. Small aggregates of large spindle-shaped cells that demonstrate moderate anisocytosis, anisokaryosis, and variable nucleus/cytoplasm ratio are the predominant cell type. The cytoplasm is clear to moderately basophilic and the wispy cytoplasmic tails have indistinct cytoplasmic borders. The cytologic findings are indicative of a sarcoma. A hemangiosarcoma was diagnosed histologically. The surrounding erythrocytes accentuate the large size of the neoplastic cells.



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## 18 Case Histories

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*To study the phenomenon of disease without books is to sail an uncharted sea, while to study books without patients is not to go to sea at all.*

Sir William Osler, MD

*1849-1919 (pathologist, physician, teacher, historian, and bibliographer of medicine; considered the "father" of veterinary pathology: first to teach pathology to veterinary students)*

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### 18.1 CASE 3-1

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**Patient:** German shepherd dog, spayed female, 10 years of age

**History:** Urinary incontinence began about a month ago. The referring veterinarian treated the dog with diethylstilbestrol (DES), increasing the dosage to 3 mg PO SID when the incontinence persisted. A single treatment of estradiol cypionate (ECP) of unknown dose was also given 2½ weeks before referral. The animal was referred with a primary complaint of blood-stained perineal area.

**PE:** Slightly depressed with normal hydration, mucous membrane color, pulse rate, respiratory rate, and temperature. Moderate dental tartar and gingivitis were present. An open wound was present on the lateral aspect of the left stifle. Isolated ecchymotic hemorrhages were observed on the ventral abdomen

#### 18.1.1 NOTABLE LABORATORY FINDINGS

**Hematology:** HCT = 23% with normal RBC indices, platelet count = 6000/μL, neutrophil count = 500/μL, lymphocyte count = 2000/μL, monocyte count = 100/μL, eosinophil count = 0/μL, RBC morphology = 1+ echinocytosis and moderate rouleaux, lymphocyte morphology = frequently reactive

**Clinical chemistry:** Not done.

**Bone marrow:** Aspirate and core biopsies were markedly hypocellular. The marrow particles present in aspirate smears consisted primarily of reticular cells, macrophages, plasma cells, and mast cells. Low numbers of erythroid precursor cells and even lower numbers of granulocytic precursor cells were present. No megakaryocytes were observed. Large amounts of stainable iron were present.

#### 18.1.2 ASSESSMENT

The anemia in this dog was considered nonregenerative because no polychromasia was seen in the stained blood film. The reactive lymphocytes indicated increased antigenic stimulation, probably related to the profound neutropenia and open wound. The subcutaneous hemorrhages resulted from the thrombocytopenia. Bone marrow evaluation revealed that the pancytopenia present resulted from a lack of bone marrow precursor cells. When erythroid precursors, granulocyte precursors, and megakaryocytes are markedly reduced or absent, the term *aplastic anemia* is used. The lymphocyte count was normal because most lymphocytes in blood enter from lymph nodes rather than from the bone marrow. The reticular cells, macrophages, and plasma cells were

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considered to be normal residual cells. Mast cells are rare in bone marrow of normal animals but are sometimes seen in aplastic bone marrow, possibly because microenvironment changes potentiate their development.

## 18.1.3 COMMENT

The dog was given a blood transfusion and antibiotic therapy was begun. Epistaxis began 5 days later. The HCT was 27%. A bone marrow aspirate collected at that time was again aplastic. A second blood transfusion was given. Marked hematuria occurred 3 days later and the owner decided to have the dog euthanized. The aplastic anemia in this dog resulted from the prior administration of high doses of estrogens. ECP is much more toxic to canine bone marrow than is DES. Experimental studies have indicated that aplastic anemia develops about 3 weeks after toxic doses of estrogen are given, in agreement with the time course of this case. Only dogs and ferrets have been reported to develop estrogen-induced aplastic anemia. In addition to iatrogenic estrogen toxicity, dogs with endogenous hyperestrogenism (Sertoli cell tumor, interstitial cell tumor, and seminoma of the testicle; and granulosa cell tumor of the ovary) can develop aplastic anemia, as can ferrets with protracted estrus.

## 18.1.4 References

GJ Kociba, CA Caputo: Aplastic anemia associated with estrus in pet ferrets. *J Am Vet Med Assoc.* **178**, 1981, 1293–1294.

N Miura, N Sasaki, H Ogawa, et al.: Bone marrow hypoplasia induced by administration of estradiol benzoate in male beagle dogs. *Jpn J Vet Sci.* **47**, 1985, 731–739.

RV Morgan: Blood dyscrasias associated with testicular tumors in the dog. *J Am Anim Hosp Assoc.* **18**, 1982, 970–975.

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## 18.2 CASE 3-2

**Patient:** Quarter horse, gelding, 15 years of age

**History:** Intermittent unilateral epistaxis for 4 months and weight loss for 2 months

**PE:** Pale mucous membranes, tachycardia, temperature = 103.8°F

### 18.2.1 NOTABLE LABORATORY FINDINGS

**Hematology:** HCT = 12% with normal RBC indices, fibrinogen = 1000 mg/dL, platelets = 142,000/μL, neutrophil count = 400/μL, lymphocyte count = 2200/μL, monocyte count = 100/μL, RBC morphology = 2+ anisocytosis, lymphocyte morphology = 5% of lymphocytes were moderately large with fine nuclear chromatin and scant basophilic cytoplasm

**Urinalysis:** Normal.

**Clinical chemistry:** Unremarkable

**Coggins' test:** Negative

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*Coagulation tests:* PT and APTT normal

*Platelet function tests:* Normal platelet aggregation and normal vWF concentration

*Bone marrow biopsy:* Replacement of normal marrow cells with a monotonous population of moderately large lymphocytes with fine nuclear chromatin and scant basophilic cytoplasm similar to those present in blood. Indistinct nucleoli were visible in some cells. As expected, these neoplastic cells were peroxidase negative.

*Endoscopy:* Normal nasopharynx, guttural pouch, and upper trachea

## 18.2.2 ASSESSMENT

In the absence of identifiable peripheral tumors, a presumptive diagnosis of acute lymphoblastic leukemia was made. The severe neutropenia and anemia were explained by the replacement of normal marrow precursor cells with neoplastic cells. The normal platelet count was unexplained, as was the long history of intermittent epistaxis. The increased fibrinogen concentration suggested the presence of concomitant inflammation.

## 18.2.3 COMMENT

The horse was euthanized. Multiple submucosal hematomas were present in the maxillary sinuses, and mesenteric and sublumbar lymph nodes were enlarged. As in the antemortem biopsy, the bone marrow was diffusely filled with sheets of neoplastic lymphocytes. Lymphoid infiltrates were present in the spleen, liver, kidney, and lymph nodes. Moderate extramedullary hematopoiesis was present in the spleen; consequently, some of the blood platelets may have originated in the spleen. It is also possible that some bone marrow sites, not evaluated during the antemortem biopsy or necropsy, contained megakaryocytes. The necropsy findings were more supportive of a diagnosis of acute lymphoblastic leukemia than of a diagnosis of lymphoma with secondary leukemia.

## 18.2.4 References

GD Lester, AR Alleman, RE Raskin, et al.: Pancytopenia secondary to lymphoid leukemia in three horses. *J Vet Intern Med.* 7, 1993, 360–363.

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## 18.3 CASE 4-1

**Patient:** Irish setter dog, female, 13 years of age

**History:** Weakness, anorexia, and weight loss for a week, and unable to walk at presentation

**PE:** The dog was depressed, emaciated, approximately 8% dehydrated, and nonambulatory because of extreme weakness. Pale mucous membranes, ocular and nasal discharges, excessive dental tartar, otitis externa, and large numbers of fleas were present. The rectal temperature was 99.6°F

### 18.3.1 NOTABLE LABORATORY FINDINGS

*Hematology:* HCT = 11%; MCV = 52 fL; MCHC = 30 g/dL; RDW = 20%; reticulocyte count = 80,000/μL; total plasma protein = 6.8 g/dL; platelet count = 532,000/μL; band count = 3300/μL; neutrophil count =

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24,800/ $\mu$ L; lymphocyte count = 500/ $\mu$ L; monocyte count = 3600/ $\mu$ L; eosinophil count = 0; RBC morphology = 1+ polychromasia, 2+ anisocytosis, 2+ hypochromasia

*Clinical chemistry:* BUN = 29 mg/dL, creatinine = 1.0 mg/dL

*Serum iron assays:* Serum iron = 16 mg/dL (reference range 84 to 233  $\mu$ g/dL), total iron-binding capacity (TIBC) = 462  $\mu$ g/dL, and ferritin = 140  $\mu$ g/L (reference range 80 to 800  $\mu$ g/L)

*Fecal flotation:* *Trichuris* eggs

### 18.3.2 ASSESSMENT

The presence of a severe microcytic hypochromic anemia indicates the presence of chronic iron deficiency. The low serum iron, normal serum TIBC, and low-normal serum ferritin concentrations support the diagnosis of iron deficiency. Serum ferritin concentration generally correlates well with total body iron content, but ferritin is an acute-phase reactant protein that increases during inflammation. Consequently, serum ferritin might have been lower in the absence of the inflammation documented in the physical examination. Iron deficiency is almost always the result of blood loss in adult animals. The massive flea infestation was believed to be the major source of blood loss in this dog. Some blood loss may have also occurred in the feces, but whipworms alone do not cause enough hemorrhage to result in iron deficiency anemia. The increased RDW indicates that there is increased variation in RBC volumes present. In iron deficiency anemia, this results from a mixture of normocytic RBCs and microcytic RBCs formed after iron becomes limiting for RBC development. The absolute reticulocyte count was not increased, indicating that decreased iron availability is limiting the bone marrow response to the anemia. The normal total plasma and serum protein concentrations in a dehydrated animal suggests that the concentration will be low-normal or decreased after rehydration. Serum proteins are synthesized more rapidly than RBCs; consequently, the total plasma protein concentration may be normal in animals with chronic blood loss. A majority of dogs with iron deficiency anemia have a thrombocytosis, as was present in this case. The neutrophilia, lymphopenia, monocytosis, and eosinopenia are likely the result of stress (endogenous glucocorticoid release), but the significant left-shift and the magnitude of the neutrophilia indicates a concomitant inflammatory response is also present. The slightly increased BUN concentration is probably prerenal and secondary to dehydration.

### 18.3.3 COMMENT

The dog was given a whole blood transfusion (two units) and treated with intravenous lactated Ringer's solution to correct the dehydration. The following day the HCT was 34%, total plasma protein was 6.2 g/dL, rectal temperature was 101.5°F, and marked clinical improvement was apparent. The animal was also given a flea bath and treated with an anthelmintic, and the client was instructed on appropriate flea control measures for the dog's environment. Oral iron therapy was not considered essential because of the amount of iron present in the transfused blood.

### 18.3.4 References

JW Harvey, TW French, DJ Meyer: Chronic iron deficiency anemia in dogs. *J Am Anim Hosp Assoc.* **18**, 1982, 946–960.

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## 18.4 CASE 4-2

**Patient:** Domestic short-hair cat, castrated male, 2 years of age

**History:** Presented for evaluation of deformed carpus, which was present when the client acquired the cat as a stray

**PE:** Deformity of carpus secondary to traumatic luxation, alopecia over pinna secondary to dermatomycosis, slightly depressed and afebrile with marked splenomegaly

### 18.4.1 NOTABLE LABORATORY FINDINGS

**Hematology:** HCT = 13%; MCV = 86 fL; MCHC = 33% total plasma protein = 8.3 g/dL; platelet count = normal; leukocyte counts = normal; nucleated RBCs = 1400/ $\mu$ L; RBC morphology = 1+ anisocytosis, 2+ polychromasia, 4+ *Mycoplasma haemofelis* organisms

**Clinical chemistry:** Bilirubin = 0.4 mg/dL, ALT = 143 U/L, globulin = 5.8 g/dL

**Serology:** FeLV = negative, FIV = positive

### 18.4.2 ASSESSMENT

The anemia present was regenerative based on the degree of polychromasia present. Reticulocyte counts may not be accurate when high numbers of *M. haemofelis* organisms are present. The macrocytosis and nucleated RBCs are consistent with the regenerative bone marrow response present. The increased total plasma protein concentration is the result of increased globulin concentrations and could represent an inflammatory reaction to the blood parasite. The slightly increased bilirubin concentration is attributable to the increased RBC destruction that accompanies these RBC parasites. The slightly increased ALT may reflect hypoxic injury to the liver.

### 18.4.3 COMMENT

Doxycycline and glucocorticoid therapy was initiated and the cat was discharged. The client was told that the *M. haemofelis* infection should respond to therapy, but that the cat would probably remain FIV positive, which would likely result in increased susceptibility to bacterial infections at a later date. Concurrent infections of *M. haemofelis* and FeLV generally result in more severe clinical signs and more severe anemia than occurs when a cat is infected with either agent alone. In contrast, concurrent infection with *M. haemofelis* and FIV does not appear to cause more severe anemia than does infection with *M. haemofelis* alone. Consequently, the regenerative anemia in this cat is attributable primarily to the *M. haemofelis* infection.

### 18.4.4 References

JW Harvey: Hemotropic mycoplasmosis. In Greene, CE (Ed.): *Infectious diseases of the dog and cat*. ed 3., 2005, WB Saunders, St. Louis, (in press).

## 18.5 CASE 5-1

**Patient:** Standardbred horse, female, 10 years of age

**History:** Dystocia resulting in a vaginal tear and displacement of intestines into the vagina. Attempts to repair the laceration on the farm were initially unsuccessful because of hemorrhage and straining. Xylazine was administered as an analgesic and sedative, the intestines were replaced into the abdomen, and the laceration was sutured.

**PE:** The horse was uncomfortable, exhibiting evidence of pain, but otherwise appeared normal

### 18.5.1 NOTABLE LABORATORY FINDINGS (Day 1)

*Hematology:* HCT = 38%, total plasma protein = 6.0 g/dL, fibrinogen = 300 mg/dL, platelet count = normal, metamyelocyte count = 100/μL, band count = 600/μL, neutrophil count = 2400/μL, lymphocyte count = 600/μL, monocyte count = 200/μL, eosinophil count = 0/μL, and neutrophilic morphology = 2+ toxicity

*Clinical chemistry:* AST = 506 U/L

*Abdominal fluid:* HCT = 8%, protein = 4.0 g/dL, nucleated cell count = 13,100/μL, most nucleated cells present were toxic neutrophils

*Microbiology:* No bacterial growth was obtained from the abdominal fluid, but antibiotic therapy may have been initiated prior to culture.

### 18.5.2 ASSESSMENT

The abdominal fluid analysis revealed evidence of hemorrhage and inflammation. The toxic left shift with low-normal neutrophil numbers in blood resulted from peritonitis with movement of neutrophils into the abdominal cavity. The absorption of endotoxin, which results in increased margination of neutrophils, may also have contributed to this leukogram. The lymphopenia and eosinopenia probably resulted from the endogenous release of glucocorticoids. The slightly decreased plasma protein concentration probably resulted from the peritonitis with protein movement into the abdominal cavity. The increased serum AST activity was attributed to tissue injury.

### 18.5.3 COMMENT

The abdomen was lavaged with large volumes of saline solution containing penicillin and streptomycin and the horse was treated with intravenous penicillin and intravenous fluids. Laboratory analyses were done again on day 3

### 18.5.4 NOTABLE LABORATORY FINDINGS (Day 3)

*Hematology:* HCT = 42%, total plasma protein = 7.1 g/dL, fibrinogen = 700 mg/dL, platelet count = normal, band count = 200/μL, neutrophil count = 900/μL, lymphocyte count = 300/μL, monocyte count = 200/μL, eosinophil count = 0/μL, and neutrophilic morphology = 2+ toxicity

*Clinical chemistry:* AST = 795 U/L

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*Abdominal fluid:* HCT = 3%, protein = 2.9 g/dL, nucleated cell count = 33,800/μL, most nucleated cells present were toxic neutrophils.

## 18.5.5 ASSESSMENT

The abdominal fluid analysis revealed continued evidence of inflammation. The toxic neutropenia on day 3 resulted from peritonitis with movement of neutrophils into the abdominal cavity. The lymphopenia and eosinopenia resulted from the endogenous release of glucocorticoids. The fibrinogen increased in response to inflammation. The increased serum AST activity was attributed to tissue injury. The horse eventually made a full recovery.

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## 18.6 CASE 5-2

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**Patient:** Himalayan cat, castrated male, 5 years of age

**History:** Respiratory distress developed 3 days earlier. The referring veterinarian began treatment with an antibiotic, but the condition worsened.

**PE:** The cat presented with abdominal respiration and tachypnea. Harsh lung sounds were auscultated, the cat was underweight and may have been slightly dehydrated. Enlarged prescapular, axillary, and inguinal lymph nodes and splenomegaly were palpated. Papules and scabs on the head and base of the tail were believed to represent a flea-bite allergy. The rectal temperature was 102.6°F

## 18.6.1 NOTABLE LABORATORY FINDINGS

*Hematology:* HCT = 29% with normal RBC indices, total plasma protein = 8.6 g/dL, platelet count = normal, neutrophil count = 15,600/μL, lymphocyte count = 5100/μL, monocyte count = 1200/μL, band eosinophil count = 400/μL, eosinophil count = 22,300/μL, basophil count = 2100/μL, RBC morphology = normal

*Clinical chemistry:* Total serum protein = 8.1 g/dL, total globulins = 5.9 g/dL

*Exfoliative cytology:* Transthoracic lung aspiration revealed histiocytic eosinophilic inflammation. Increased numbers of eosinophils were also present in splenic and lymph node aspirates that appeared to exceed those present in contaminating blood.

*Histopathology:* Chronic ulcerative eosinophilic dermatitis

*Parasitology:* ELISA heartworm test was negative.

*Thoracic radiograph:* Patchy interstitial infiltrate

*Abdominal ultrasound:* Splenomegaly and slightly thickened loops of bowel

## 18.6.2 ASSESSMENT

Based on the magnitude of the eosinophilia and evidence of eosinophilic infiltration and injury in multiple organs, a diagnosis of hypereosinophilic syndrome was made. The etiology of this syndrome is unknown. Evidence that the overproduction of IL-5 may be involved in producing this disorder has been presented in people with hypereosinophilic syndrome. A marked left shift in the eosinophilic series is expected in cats with

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eosinophilic leukemia. Eosinophilic leukemia was considered unlikely in this cat, because most of the eosinophils in blood and tissues were mature. When present in animals, basophilia generally accompanies eosinophilia, possibly because certain growth factors (most notably IL-5) stimulate the production of both cell types. The slight neutrophilia and monocytosis present may be associated with the inflammation recognized in several tissues. The mild nonregenerative anemia is probably the result of the anemia of inflammatory disease. The increased serum protein concentration was the result of increased globulins, further supporting the likelihood of an inflammatory reaction.

### 18.6.3 COMMENT

The cat was placed in an oxygen cage and treated with aminophylline (a bronchodilator) and an antibiotic pending the outcome of diagnostic tests. Once a diagnosis of hypereosinophilic syndrome was reached, glucocorticoid therapy was initiated. Clinical signs improved rapidly and the cat was discharged with a plan to taper the glucocorticoid dosage as clinical signs resolved.

### 18.6.4 References

BA Huibregse, JL Turner: Hypereosinophilic syndrome and eosinophilic leukemia: a comparison of 22 hypereosinophilic cats. *J Am Anim Hosp Assoc.* **30**, 1994, 591–599.

CL Swenson, MA Carothers, ML Wellman, et al.: Eosinophilic leukemia in a cat with naturally acquired feline leukemia virus infection. *J Am Anim Hosp Assoc.* **29**, 1993, 467–501.

PF Weller, GJ Bubley: The idiopathic hypereosinophilic syndrome. *Blood.* **83**, 1994, 2759–2779.

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### 18.7 CASE 6-1

**Patient:** Rottweiler dog, female, 2 years of age

**History:** Depression, lethargy, fever, and purulent bloody vaginal discharge for several days; vomited the day of admission

**PE:** Moderately depressed, panting respiration, slightly distended abdomen, dark-pink mucous membranes, rectal temperature 102 °F

#### 18.7.1 NOTABLE LABORATORY FINDINGS (Day 1)

**Hematology:** HCT = 48%, total plasma protein = 7.5 g/dL, fibrinogen = 200 mg/dL, manual platelet count = 180,000/μL, band neutrophil count = 3700/μL, neutrophil count = 57,400/μL, lymphocyte count = 7700/μL, monocyte count = 3700/μL, eosinophil count = 700/μL, basophil count = 0/μL, RBC morphology = 1+ echinocytes, leukocyte morphology = 1+ toxicity of neutrophilic cells and occasional reactive lymphocytes

**Clinical chemistry:** Unremarkable

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*Coagulation tests:* Prothrombin time (PT) = 9 seconds (control = 8 seconds), activated partial thromboplastin time (APTT) = 20 seconds (control = 10 seconds), activated clotting time (ACT) = 105 seconds (reference <120 seconds), fibrin degradation products (FDP) = positive at 1:20 dilution

*Abdominal radiographs:* No abnormalities appreciated

*Abdominal ultrasound:* Large, fluid-filled uterus identified

### 18.7.2 ASSESSMENT

The marked neutrophilia with toxic left shift and monocytosis indicated a severe inflammatory reaction. The presence of a dilated uterus and purulent vaginal discharge indicated that the dog had pyometra. The lymphocytosis may have reflected antigenic stimulation. The decreased platelet count, prolonged APTT, and positive FDP test indicated the presence of DIC. Although the PT was normal, this test appears to be less sensitive than the APTT in the diagnosis of DIC. The ACT may have been normal because the ACT is less sensitive than the APTT in revealing abnormalities of the intrinsic and common coagulation pathways. Fibrinogen is an acute-phase protein that tends to increase during inflammation; consequently, the normal fibrinogen value did not rule out DIC.

### 18.7.3 COMMENT

Antibiotic and intravenous fluid therapy was begun after the first blood sample was taken. Epistaxis began on the following day and the animal became more depressed

### 18.7.4 NOTABLE LABORATORY FINDINGS (Day 2)

*Hematology:* HCT = 32% with normal RBC indices, total plasma protein = 6.4 g/dL, fibrinogen = 400 mg/dL, manual platelet count = 61,000/ $\mu$ L, metamyelocyte count = 800/ $\mu$ L, band neutrophil count = 8400/ $\mu$ L, neutrophil count = 65,900/ $\mu$ L, lymphocyte count = 2100/ $\mu$ L, monocyte count = 7200/ $\mu$ L, eosinophil count = 0/ $\mu$ L, basophil count = 0/ $\mu$ L, RBC morphology = 1+ echinocytes, leukocyte morphology = 1+ toxicity of neutrophilic cells and occasional reactive lymphocytes

*Coagulation test:* ACT = 150 seconds (reference <120 seconds)

### 18.7.5 ASSESSMENT

The neutrophilia with toxic left shift and monocytosis were more pronounced on the second day. The decrease that occurred in lymphocyte count and eosinopenia that developed suggested that increased endogenous glucocorticoid release occurred as the dog's clinical condition worsened. The decreased HCT on the second day was primarily the result of fluid therapy. The further decrease in the platelet count and the prolonged ACT suggested the continuation of DIC.

### 18.7.6 COMMENT

Ovariohysterectomy was performed after the blood sample was collected on the second day and the dog made an uneventful recovery.

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## 18.7.7 References

E Sevelius, A Tidholm, K Thoren-Tolling: Pyometra in the dog. *J Am Anim Hosp Assoc.* 26, 1990, 33–38.

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## 18.8 CASE 7-1

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**Patient:** Cocker spaniel dog, male, 4 years of age

**History:** Rear-leg lameness associated with bilateral hip dysplasia was diagnosed 2 years ago, and erosive nonseptic arthritis involving the carpal and tarsal joints was recognized 4 months ago. The HCT and platelet counts were normal at that time, but the ANA test was positive at 1:100 dilution (reference <1:20). The dog has been treated with aspirin the past 4 months.

**PE:** The dog was depressed with pale mucous membranes. A polyarthropathy was present and all joints were painful. There were multiple raised pigmented skin lesions and small petechial hemorrhages were present on the penis and abdomen. The rectal temperature was normal

### 18.8.1 NOTABLE LABORATORY FINDINGS

**Hematology:** HCT = 23%; MCV = 74 fL; MCHC = 33 g/dL; reticulocyte count = 184,000/μL; total plasma protein = 7.9 g/dL; fibrinogen = 400 mg/dL; manual platelet count = 8000/μL; band neutrophil count = 600/μL; neutrophil count = 12,900/μL; lymphocyte count = 700/μL; monocyte count = 200/μL; eosinophil count = 1000/μL; nucleated RBC count = 500/μL; RBC morphology = 3+ anisocytosis, 2+ polychromasia, 3+ spherocytosis, occasional Howell-Jolly bodies, and autoagglutination of saline washed RBCs

**Clinical chemistry:** Total serum protein = 8.2 g/dL and the total globulin = 5.6 g/dL

**Urinalysis:** Specific gravity = 1.042, moderate bilirubinuria

**Joint fluid:** A direct smear from a swollen joint revealed increased numbers of nondegenerate neutrophils and macrophages.

**ANA test:** Positive at 1:320 dilution (reference <1:20)

**Skin biopsy:** Histologic lesions were consistent with pemphigus foliaceus, an immune-mediated skin disorder, but direct immunofluorescence examination for IgG deposits in skin was negative.

### 18.8.2 ASSESSMENT

The presence of autoagglutination of saline-washed RBCs and spherocytosis indicates the presence of an immune-mediated anemia. The high-normal MCV and slightly decreased MCHC result from the increased percentage of reticulocytes present and the absolute reticulocytosis indicates an appropriate bone marrow response to the anemia. The low number of nucleated RBCs present is suitable for the degree of reticulocytosis. The petechial hemorrhages present can be attributed to the severe thrombocytopenia. Based on the presence of an immune-mediated hemolytic anemia and a positive ANA test, the thrombocytopenia was presumed to be immune mediated. The combined presence of immune-mediated anemia and

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thrombocytopenia has been termed the “Evans' syndrome.” The presence of high-normal numbers of eosinophils suggests that endogenous glucocorticoid release is not responsible for the neutrophilia, monocytosis, and lymphopenia. The increased total globulins in serum and high-normal plasma fibrinogen concentration are consistent with inflammation, as in the mild neutrophilia with left shift and monocytosis. Bilirubinuria is common in dogs with hemolytic anemia even when bilirubinemia is not present, because of the low renal threshold for bilirubin in dogs. A presumptive diagnosis of systemic lupus erythematosus (SLE) was made based on the concomitant occurrence of immune-mediated hemolytic anemia, thrombocytopenia, nonseptic polyarthritis, and positive ANA test. The skin lesion may also have been a component of this syndrome, but an immune-mediated etiology could not be confirmed.

### 18.8.3 COMMENT

Therapy consisted of glucocorticoid steroids and cyclophosphamide. When examined 1 week later, the animal appeared less painful, skin lesions were resolving, the HCT was 27%, MCV was 77 fL, and platelet count was  $1.2 \times 10^6/\mu\text{L}$ . The resolution of the thrombocytopenia following initiation of immunosuppressive therapy provides retrospective evidence that the thrombocytopenia was immune mediated. It is assumed that the animal had high plasma thrombopoietin values when thrombocytopenic and that the subsequent thrombocytosis occurred as a rebound phenomenon when premature platelet destruction was reduced or eliminated by immunosuppressive therapy.

### 18.8.4 References

CB Grindem, KH Johnson: Systemic lupus erythematosus: literature review and report of 42 new canine cases. *J Am Anim Hosp Assoc.* **19**, 1983, 489–503.

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### 18.9 CASE 10-1

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**Patient:** Mixed-breed dog, female, 4 years of age

**History:** Dog had been showing signs of estrus; was gone all night; returned the next day; vomited “garbage” 2 to 3 times; taken to the veterinarian the next day.

**PE:** Depressed, 6% to 8% dehydrated, fluid- and gas-filled intestinal tract

**Radiology:** Unremarkable

#### 18.9.1 NOTABLE LABORATORY FINDINGS (Initial)

**Hematology:** HCT = 49%, plasma protein = 7.7 g/dL, neutrophil count = 18,800/ $\mu\text{L}$ , lymphocyte count = 600/ $\mu\text{L}$ , platelet count = 220,000/ $\mu\text{L}$

**Chemistries:** ALT = 1340 U/L, AST = 655 U/L, ALP = 155 U/L, urea nitrogen = 41 mg/dL, creatinine = 2.1 mg/dL, total bilirubin = 1.1 mg/dL, total protein = 7.1 g/dL, albumin = 4.1 g/dL, amylase and lipase within reference range

**Urinalysis:** Specific gravity = 1.045, moderate bilirubinuria, hyaline casts (0-2 per HPF)

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### 18.9.2 ASSESSMENT

The changes in the hematocrit; plasma protein, total protein, albumin, urea nitrogen, and creatinine concentrations; urine specific gravity; and hyaline casts are a consequence of dehydration. The neutrophilia and lymphopenia are indicative of “stress.” The increased serum ALT and AST activities indicate hepatocellular injury. The hyperbilirubinemia (with bilirubinuria) and increased serum ALP activity indicate a cholestatic component; the minimal increase in the ALP activity supports a rapid onset. The increased serum urea nitrogen and creatinine concentrations coincident with a concentrated urine specific gravity indicate prerenal azotemia caused by dehydration.

### 18.9.3 COMMENT

Supportive medical management was instituted. During the third day, spontaneous bleeding was noted from the venipuncture sites; the dog appeared stuporous and was referred for further evaluation.

### 18.9.4 PE

Semi-comatose, pale mucous membranes, scleral icterus, abdominal fluid

### 18.9.5 NOTABLE LABORATORY FINDINGS

*Hematology:* HCT = 12%, plasma protein = <2 g/dL, schistocytes present, platelet count = 5000/ $\mu$ L, slightly reddish-yellow discolored plasma, prothrombin time = prolonged 20 seconds, activated partial thromboplastin time = prolonged 33 seconds, fibrin degradation products (+, >1:40)

*Chemistries:* ALT = 102 U/L, AST = 93 U/L, ALP = 41 U/L, glucose = 45 mg/dL, urea nitrogen = 4 mg/dL, creatinine = 1.5 mg/dL, total bilirubin = 3.1 mg/dL, total protein = 1.1 g/dL, albumin = 0.6 g/dL, ammonia = 125  $\mu$ g/dL (reference <40  $\mu$ g/dL)

*Abdominocentesis:* Red-colored fluid, cytologically consistent with blood

### 18.9.6 ASSESSMENT

The hematocrit and plasma protein concentration indicate blood loss; in this case there is no external hemorrhage; therefore internal bleeding is likely. The reduced serum albumin concentration cannot be due to liver disease because its plasma half-life is approximately 8 days in the dog. The hemolyzed plasma, schistocytes, thrombocytopenia, prolonged coagulation times, and presence of fibrin degradation products indicate a coagulopathy (disseminated intravascular coagulation [DIC]). The precipitous reduction in the serum ALT and AST activities suggests inadequate hepatocellular mass to maintain their increase. The reduced serum glucose and urea nitrogen concentrations (with a normal creatinine concentration) and the absence of a further increase in the serum ALP activity (in fact, a reduction), despite the presence of cholestasis, lend further support to inadequate hepatic tissue for their production. Both the hemolytic component of DIC and the cholestatic process contribute to the increased serum total bilirubin concentration. The hyperammonemia confirms the presence of hepatic encephalopathy.

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## 18.9.7 COMMENT

The dog died 6 hours later. On histologic examination, virtually no viable hepatocellular tissue was observed. Numerous pigment-filled macrophages replaced most of the parenchymal tissue. No cause was apparent. These findings are consistent with an acute toxic insult to the liver. Drugs such as mebendazole and trimethoprim-sulfa combinations can cause similar hepatic histopathologic findings. Fulminant hepatic failure is likely the cause of the coagulopathy.

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## 18.10 CASE 10-2

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**Patient:** American quarter horse, mare, 8 years of age

**History:** Acute onset of lethargy, inappetence, head-pressing, circling. She had a 2-month-old foal and had been given tetanus antitoxin at the time of parturition.

**PE:** Lethargy, stupor, jaundice, self-inflicted facial skin lesions

### 18.10.1 NOTABLE LABORATORY FINDINGS

**Hematology:** HCT = 58%, plasma protein = 8.0 g/dL, icteric plasma

**Chemistries:** Total bilirubin = 12 mg/dL, AST = 1966 U/L, SD = 445 U/L, CK = 1105 U/L, GGT = 135 U/L, total bile acid = 55  $\mu$ mol/L, plasma ammonia concentration = 25  $\mu$ g/dL (reference <40  $\mu$ g/dL), total protein = 7.6 g/dL, urea nitrogen = 48 mg/dL, creatinine = 3.1 mg/dL

### 18.10.2 ASSESSMENT

The polycythemia and increased plasma protein, serum urea nitrogen, and creatinine concentrations indicate dehydration. The increased SD and AST activities indicate hepatocellular injury. The increased serum CK activity indicates muscle injury, probably associated with the trauma. The increased serum GGT activity indicates a cholestatic component to the hepatic disease. Fasting results in hyperbilirubinemia; in this case, cholestasis may be contributing to the increased total bilirubin concentration as well because of the increased serum total bile acid concentration, which indicates hepatic dysfunction, consistent with signs of hepatic encephalopathy. The plasma ammonia concentration was within the reference range; this is not surprising, since it is labile and dependent on the ingestion of protein.

### 18.10.3 COMMENT

The histopathologic findings of a needle biopsy specimen from the liver included severe hepatocellular degeneration and parenchymal collapse (predominantly in zone 3) with canalicular/bile ductular casts and mild bile duct hyperplasia. The histopathologic findings of relatively acute hepatocellular injury involving zone 3 are consistent with the disease termed *serum hepatitis*.

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## 18.11 CASE 10-3

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**Patient:** Doberman pinscher, spayed female, 8 years of age

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**History:** Persistent, mildly increased serum hepatic enzyme levels for 1 year. Treatment with prednisolone for 1 month resulted in no change in the serum ALT activity, caused the serum ALP activity to increase, and was associated with severe polydipsia and polyuria. The client declined further treatment. The dog was active and eating well during this entire time. One month before referral, polydipsia and polyuria developed. Two weeks earlier, a distended abdomen, frequent drooling, and intermittent head shaking were observed; and the dog acted disoriented.

**PE:** Alert, ascites present

### 18.11.1 NOTABLE LABORATORY FINDINGS

*Hematology:* HCT = 40%, MCV = 58 fL, platelets adequate, prothrombin time = normal, activated partial thromboplastin time = prolonged 8 seconds (24 hours after 2 subcutaneous treatments with vitamin K<sub>1</sub>, it returned to normal)

*Chemistries:* Albumin = 2.2 g/dL, ALT = 155 U/L, ALP = 242 U/L, fasting total bile acid = 235 µmol/L. During an episode of abnormal neurologic behavior: ammonia = 160 µg/dL (reference <40 µg/dL), fasting total bile acid = 226 µmol/L. Twenty-four hours after treatment with neomycin and lactulose, ammonia = 21 µg/dL and total bile acid = 240 µmol/L.

*Urinalysis:* Specific gravity = 1.025, bilirubinuria 2+

*Abdominal fluid:* Total protein = <2.5 g/dL, low cell count, predominantly mesothelial cells with a small number of neutrophils and lymphocytes

*Ultrasound findings:* Abdominal fluid, small liver with irregular surface and variable echogenic density. An attempt to obtain a needle biopsy was not successful.

### 18.11.2 ASSESSMENT

The findings are consistent with hepatic insufficiency resulting in hepatic encephalopathy, reduced albumin and coagulation factor (activated) production, and portal hypertension causing ascites (transudate). The ammonia and total bile acid measurements demonstrate the different values obtained in assessment of the various components of hepatic function. The microcytosis, sometimes accompanied by a mild anemia, is observed in association with portosystemic shunts (congenital and acquired) for reasons that are poorly understood.

### 18.11.3 COMMENT

After 2 days of treatment with vitamin K<sub>1</sub>, neomycin, and lactulose, a laparotomy was performed to obtain a biopsy specimen from a small liver with multiple, variably sized and colored (tan to dark brown) nodules. Multiple portocaval shunts were prominent near the left kidney. The descriptive histopathologic findings included nodules of hepatocytes often vacuolated, cholangiohepatitis, bile duct proliferation, and lipogranulomas (foci of fat-filled macrophages). The findings were initially suggestive of cirrhosis. Reassessment after application of reticulin and trichrome stains demonstrated multiple nodules that compressed the existing hepatic tissue into the portal tract areas. These areas consisted of condensed reticulin tissue; mixed inflammatory cell infiltration; extramedullary hematopoiesis that included myeloid precursors, giving the additional appearance of inflammation; and bile duct proliferation. There was only minimal

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increase in the connective tissue; a classification of cirrhosis was not justified. The findings were interpreted as marked nodular proliferation (hyperplasia) with nonspecific secondary changes (Kelly, 1993; Meyer, 1996). The differentiation from cirrhosis is important because the disease is not “driven” by a necroinflammatory process. The patient with hepatic insufficiency associated with nodular hyperplasia can be treated more favorably with supportive management than the patient with cirrhosis. The polydipsia and polyuria may be associated with an abnormal “re-setting” of the hypothalamic-pituitary-adrenal axis. It has been found that dogs with either congenital or acquired portosystemic shunts often have increased basal plasma cortisol and adrenocorticotrophic hormone concentrations (dexamethasone suppressible) and abnormal vasopressin regulation with a consequent reduced urine specific gravity (Rothuizen et al., 1995).

### 18.11.4 References

WR Kelly: The liver and biliary system. 4th edition., In Jubb, KVF, Kenneedy, PC, Palmer, N (Eds.): *Pathology of domestic animals*. vol II, 1993, Academic Press, San Diego, 349.

DJ Meyer: Hepatic pathology. In Guilford, WG, Center, SA, Strombeck, DR, et al. (Eds.): *Strombeck's small animal gastroenterology*. 3rd ed., 1996, WB Saunders, Philadelphia, 633–653.

J Rothuizen, WJ Biewenga, JA Mol: Chronic glucocorticoid excess and impaired osmoregulation of vasopressin release in dogs with hepatic encephalopathy. *Domest Anim Endocrinol*. 12, 1995, 13–24.

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### 18.12 CASE 10-4

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**Patient:** Persian cat, female, 6 months of age

**History:** Mellow personality; occasional exaggerated chewing movements (like something stuck in its mouth); appears occasionally ataxic; two seizures were observed; presented in semi-comatose state

**PE:** Small for age, semi-aware of surroundings but could not be aroused

#### 18.12.1 NOTABLE LABORATORY FINDINGS

*Hematology:* HCT = 28%, MCV = 36 fL, normochromic erythrocytes with moderate numbers of poikilocytes

*Chemistries:* ALP = 125 U/L, urea nitrogen = 8 mg/dL, creatinine = 1.5 mg/dL, ammonia = 340 µg/dL (reference <40 µg/dL), fasting total bile acid = 78 µmol/L

*Urinalysis:* Ammonium biurate crystals

#### 18.12.2 ASSESSMENT

Hepatic encephalopathy is confirmed by the hyperammonemia; the reduced serum urea nitrogen concentration in combination with a normal serum creatinine concentration and the increased serum total bile acid concentration are consistent with hepatic insufficiency. The underdeveloped physical status and neurologic

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signs plus the abnormal hepatic function test results and ammonia biurate crystalluria are indicative of a congenital portosystemic shunt. A microcytic anemia, sometimes with irregularly spiculated erythrocytes, is also noted occasionally in this disorder for reasons that are poorly understood. The increased serum ALP activity is probably due to an increased activity of the bone isoenzyme associated with growth.

## 18.12.3 COMMENT

Forty-eight hours after treatment with neomycin and lactose, the cat was alert; the ammonia concentration was 35 µg/dL, and the fasting total bile acid concentration was 75 µmol/L. The reduction of the plasma ammonia concentration, along with an unchanged serum total bile acid concentration, demonstrates the value of using different types of tests for the evaluation of hepatic function. A single extrahepatic portocaval shunt was identified with contrast portography and ligated. Two weeks after surgery, the plasma ammonia concentration was 15 µg/dL and the fasting serum total bile acid concentration was 4 µmol/L.

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## 18.13 CASE 10-5

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**Patient:** Thoroughbred foal, 4 months of age

**History:** Small for age, clumsy, observed occasionally head-pressing and circling, poor appetite

**PE:** Poorly developed muscle mass, dull, depressed, unresponsive to the menace response, staggering, slightly ataxic gait, scleral icterus

## 18.13.1 NOTABLE LABORATORY FINDINGS

*Hematology:* HCT = 34%, MCV = 34 fL, normochromic erythrocytes

*Chemistries:* Total bilirubin concentration = 6.5 mg/dL, ammonia = 150 µg/dL (reference <40 µg/dL), total bile acid concentration = 76 µmol/L

## 18.13.2 ASSESSMENT

The hematocrit and MCV are appropriate for the age of the animal. Hepatic encephalopathy is confirmed by the hyperammonemia. The increased serum total bile acid concentration is indicative of liver insufficiency. The neurologic signs in a young animal with liver insufficiency are compatible with a congenital portosystemic shunt and eliminates a primary central nervous system disease as the cause of the clinical signs. The hyperbilirubinemia is probably a result of inappetence; it does not interfere with bile acid kinetics.

## 18.13.3 COMMENT

Jejunal vein contrast portography was used to identify a portocaval shunt.

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## 18.14 CASE 11-1

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**Patient:** Domestic long hair, female, spayed, 7 years of age

**History:** Vomiting 2 to 3 times a day on most days for the past 6 weeks. Most of the time the vomitus is yellow-green fluid or is slightly thicker and is the color of the canned food that it eats. Hair balls are occasionally

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observed. There are days when the cat does not act hungry. The stools have been poorly formed on occasion during the past 2 weeks. Slight weight loss has occurred.

**PE:** The body weight is 1½ pounds less than it was 8 months earlier when seen for its annual vaccinations. The cat is tense and difficult to examine

### 18.14.1 NOTABLE LABORATORY FINDINGS

*Hematology:* Neutrophil count = 16,590/μL

*Chemistries:* ALT = 155 U/L; ALP = 99 U/L; glucose = 368 mg/dL

*Urinalysis:* Glucosuria (+1)

### 18.14.2 Radiology

Examination of the abdomen is unremarkable.

### 18.14.3 ASSESSMENT

The neutrophilia and hyperglycemia (and resultant glucosuria) may be the result of stress. The findings could also be consistent with diabetes mellitus (type 1 or type 2). The raised ALT and ALP activities suggest liver disease, either primary or secondary to diabetes mellitus (lipidosis). The measurement of the serum bile acid and fructosamine concentrations are suggested along with ultrasonography to evaluate for acute pancreatitis; the measurement of the standard serum amylase and lipase activities is not diagnostically useful in the cat. The measurement of serum pancreatic lipase assayed by a feline-specific radioimmunoassay is diagnostically sensitive and specific. The owner was reluctant to pay for more tests at this time. The amount and frequency of its usual oral treatment for hair balls was increased.

### 18.14.4 Examination 2 weeks later (did not show up for its 1-week evaluation)

The frequency of the vomiting increased and it has been inappetent the past 2 days. The owner agrees to additional tests.

### 18.14.5 PE

Same as initial examination

### 18.14.6 NOTABLE LABORATORY FINDINGS

*Hematology:* Neutrophil count = 15,500/μL

*Chemistries:* ALT = 142 U/L; ALP = 95 U/L; glucose = 340 mg/dL; total bile acids (presumably fasted) = 5.5 μmol/L; fructosamine 295 μmol/L (within reference range)

*Urinalysis:* Glucosuria (+1)

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### 18.14.7 ASSESSMENT

The serum fructosamine concentration is consistent with a stress hyperglycemia as is the neutrophilia. The serum bile acid concentration is within the reference range. Extrahepatic diseases can cause abnormal hepatic tests. The patient is referred for ultrasonography of the pancreas and endoscopy.

### 18.14.8 COMMENT

Ultrasonography of the abdomen was unremarkable. Visual examination of the duodenum was unremarkable. Histopathologic findings from multiple biopsy specimens were consistent with lymphocytic-plasmacytic enteritis (moderate severity).

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### 18.15 CASE 12-1

**Patient:** German shepherd, spayed female, 5 years of age

**History:** Weakness, lethargy, inappetence, weight loss, and vomiting (unrelated to meals) present for approximately 2 months; more pronounced periodically in the past several weeks. Vitamin supplements and herbal medications prescribed at another veterinary hospital did not seem to help.

**PE:** Lethargic and thinner than expected for size

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### 18.15.1 NOTABLE LABORATORY FINDINGS

*Hematology:* HCT = 34%, reticulocyte count = 39,000/ $\mu$ L

*Chemistries:* Urea nitrogen = 32 mg/dL, creatinine = 1.4, glucose = 63 mg/dL, ALT = 86 U/L, ALP = 210, cholesterol = 99 mg/dL, albumin = 2.3 mg/dL, total protein = 7.0 mg/dL

*Urinalysis:* Specific gravity = 1.028

### 18.15.2 ASSESSMENT

There is a normocytic, normochromic, nonregenerative anemia (mild). There is a mild azotemia (with the serum creatinine concentration within the reference range) and moderately concentrated urine consistent with prerenal azotemia. However, with the protracted history of lack of oral intake of adequate food or water, one might expect the urine specific gravity to be increased more than it is. Consequently, early primary renal disease should remain as a differential diagnosis, especially with the presence of a nonregenerative anemia and a reduced muscle mass, that could affect the plasma creatinine concentration. The serum ALT and ALP activities are slightly increased and the serum albumin concentration is slightly reduced, suggestive of a hepatopathy. Hypocholesterolemia and hypoglycemia can be associated with congenital portosystemic shunts, one cause of hepatic insufficiency. Consequently, the serum bile acid concentration should be measured. A value of 6.5  $\mu$ mol/L (presumably fasted) was determined the next day, eliminating hepatic insufficiency as the primary disease process.

The constellation of nonspecific, persistent clinical signs and multiorgan system involvement is suggestive of hypoadrenocorticism (glucocorticoid deficiency only). Because mineralocorticoid secretion is not affected, the plasma sodium and potassium concentrations remain within the reference ranges. The basal plasma cortisol

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concentration was 1.2 µg/dL, and the 2-hour post-ACTH stimulation concentration was 1.6 µg/dL. The absence of a notable increase supports the diagnosis. The clinical response to glucocorticoid treatment was rapid and maintained.

### 18.15.3 COMMENT

Although endogenous glucocorticoids affect all organ systems in health, we do not understand all the reasons for the changes in the clinicopathologic measurements when there is a deficiency. They have a positive effect on hematopoiesis and gluconeogenesis and enhance the response of the renal collecting tubules to antidiuretic hormone (vasopressin). A deficiency could result in the nonregenerative anemia, hypoglycemia, and inappropriate urine specific gravity. Lack of adequate nutrition may be the cause of the hypoalbuminemia. The hypocholesterolemia may be a result of inadequate nutrition. The reason for the abnormal serum hepatic enzyme activities is not known but has been associated with hypoadrenocorticism.

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### 18.16 CASE 12-2

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**Patient:** Golden retriever, spayed female, 9 years of age

**History:** Progressive lethargy, thinning of the hair coat, and weight gain for about 6 to 8 months

**PE:** Generalized thinning of the hair coat, especially near the base of the tail; hair felt dry and coarse; skin felt slightly thickened

### 18.16.1 NOTABLE LABORATORY FINDINGS

*Hematology:* HCT = 33%, reticulocyte count = 28,000/µL

*Chemistries:* ALP = 547 U/L, cholesterol = 482 mg/dL, total T<sub>4</sub> = 1.3 µg/dL

### 18.16.2 ASSESSMENT

The history, findings on physical examination, and laboratory findings of a mild, nonregenerative anemia and hypercholesterolemia are consistent with hypothyroidism. However, the serum total thyroxine (T<sub>4</sub>) value is not supportive of the diagnosis. Because there are clinicopathologic findings indicative of reduced thyroid function, determinations of the free T<sub>4</sub> (fT<sub>4</sub> by equilibrium dialysis method) and thyroid-stimulating hormone (TSH) concentrations were performed on the same serum sample. The fT<sub>4</sub> concentration was reduced (0.4 ng/dL) and the TSH concentration was increased (72.5 ng/mL), confirming the diagnosis of *primary* hypothyroidism. The advent of validated methods for the accurate measurement of these analytes has improved the sensitivity of diagnosing hypothyroidism without the use of provocative stimulation testing strategies. The fT<sub>4</sub> component is the small fraction that enters the cell and is converted to the biologically active T<sub>3</sub>. Consequently, its reduction is a signal to the anterior pituitary for the release of TSH. As in this case, the pituitary can respond but the diseased thyroid gland cannot. Secondary hypothyroidism is indicated if both the fT<sub>4</sub> and TSH concentrations are reduced.

There is an association of increased serum ALP activity and CK activity in some cases of hypothyroidism. The mechanism is not known.

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## 18.17 CASE 13-1

**Patient:** Lhasa apso, female, 18 months of age

**History:** Marked increase in water intake during the past 6 months; occasionally urinated in the house as a puppy but now frequently voids large volumes during the night; not interested in eating for the past 2 weeks; difficulty breathing for 2 to 3 days.

**PE:** Quiet; mucous membranes are slightly pale; 6% to 8% dehydrated; mild dyspnea observed in the waiting room, which was amplified by the exertion of handling; possible decreased lung sounds on the right side

### 18.17.1 NOTABLE LABORATORY FINDINGS

*Hematology:* Hct = 29%, MCV = 64 fL, reticulocyte count = 45,000/ $\mu$ L, plasma protein = 5.1 g/dL

*Chemistries:* Urea nitrogen = 126 mg/dL, creatinine = 6.5 mg/dL, total protein = 5.3, albumin = 2.2 g/dL, cholesterol = 395 mg/dL, calcium = 7.7 mEq/L (corrected, 9.0 mEq/L), phosphorus = 11.5 mg/dL, total carbon dioxide = 11 mEq/L

*Urinalysis:* Specific gravity = 1.009, protein = 4+, casts (course granular, waxy) = 2 to 5 per HPF (reference <1 hyaline or fine granular per HPF), urine protein/creatinine ratio = 4.4 (reference <0.4)

*Radiographic findings:* Abdomen—both kidneys are small; thorax—possible increased interstitial pattern on the right side.

### 18.17.2 ASSESSMENT

The laboratory findings are indicative of chronic renal insufficiency. The nonregenerative anemia is due to a deficiency of erythropoietin. The markedly increased serum urea nitrogen, creatinine, and phosphorus concentrations reflect an insufficient glomerular filtration rate; and the marked proteinuria develops when the glomeruli can no longer retain larger molecules ( $M_r$  greater than approximately 40,000;  $M_r$  of albumin is 66,000). The reduced serum albumin concentration is a consequence of increased loss. The loss of antithrombin III ( $M_r$  65,000) can predispose to hypercoagulability and thromboembolism. The pulmonary vasculature is a common site for thromboembolism, and it is probably the cause of dyspnea in this dog. The lesion is difficult to detect on radiographs early in its development.

The specific gravity indicates that the tubules can neither concentrate nor dilute urine in response to antidiuretic hormone (ADH). Decreased tubular function contributes to the hyperphosphatemia in concert with secondary hyperparathyroidism in the more chronic cases. The reduction of viable renal tissue results in a decreased formation of calcitriol, the active form of vitamin D. Calcitriol provides negative feedback inhibition of the synthesis parathyroid hormone (PTH).

The reduced total carbon dioxide indicates metabolic acidosis, a common consequence of chronic renal failure caused by the tubules' decreased ability to excrete hydrogen ion and regenerate bicarbonate. The acidemia causes more of the albumin-bound calcium to exist in the physiologically active ionized state, which explains the absence of clinical signs of hypocalcemia when the total calcium concentration is reduced. Care should be taken not to induce rapid alkalization in these patients, since it causes a "shift" of the ionized calcium back to the bound form and could precipitate hypocalcemic tetany. The granular and waxy casts form in the

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damaged, poorly functioning tubules (with reduced filtrate flow) from the large quantities of “escaped” proteins together with the degenerating cells.

The hypercholesterolemia is probably related to the loss of albumin (and possibly other proteins). A persistent reduction in the plasma albumin concentration signals the liver to increase its production of albumin. The stimulus for enhanced albumin synthesis appears to nonspecifically activate other synthetic pathways, including those involved in the formation of lipoproteins. The cholesterol-rich particles contribute to the increased serum cholesterol concentration.

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### 18.18 CASE 15-1

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**Patient:** Golden retriever, male, 3 years of age

**History:** Reluctant to chase the Frisbee for 3 weeks, walks stiff legged, remains alert and has a good appetite, at least two previous episodes during the past 3 months that resolved within 1 week with aspirin treatment

**PE:** Alert, has short, choppy gait, no neck pain, manipulation of the stifle joints causes discomfort

#### 18.18.1 NOTABLE LABORATORY FINDINGS

*Hematology:* Neutrophil count = 17,430/ $\mu$ L

*Chemistries:* AST = 178 U/L (ALT normal); based on these results a CK was requested from the same sample; CK = 1253 U/L

*Synovial fluid:* Samples from both stifle and both carpal joints were examined cytologically. Specimens from all joints had slightly to markedly increased nucleated cell numbers (estimated to be 10,000/ $\mu$ L to >50,000/ $\mu$ L) comprised predominantly of neutrophils, no bacteria were observed.

#### 18.18.2 ASSESSMENT

The neutrophilia indicates an inflammatory process. The raised serum CK activity and neutrophil infiltration of the joints are indicative of myositis and synovitis, respectively.

#### 18.18.3 COMMENT

Radiographs of the joints were unremarkable. Multiorgan disease is suggestive of an immunemediated process. An antinuclear antibody (ANA) tests was 1:640 (reference <1:20), supportive of an immune-mediated disease. The dog was successfully managed with prednisolone. Nonerosive polyarthritis is generally found in large-breed dogs. The concurrence of myositis is uncommon.

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## 19 Algorithms

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*It is common error to infer that things which are consecutive in order of time have necessarily the relations of cause and effect*

**Jacob Bigelow**

1. Anemia, regenerative
2. Anemia, poorly regenerative or nonregenerative
3. Erythrocytosis
4. Leukopenia
5. Leukocytosis or normal leukocyte count with abnormal differential
6. Platelet/coagulation factor deficiencies
7. Hepatic test abnormalities with moderate to markedly increased aminotransferases
8. Hepatic test abnormalities with normal to slightly increased aminotransferases
9. Hematologic, biochemical, and uroanalytical findings indicative of hepatic disease
10. Total protein/albumin/globulin, increase/decreased
11. Urea nitrogen/creatinine, increased
12. Urea nitrogen/creatinine normal, with abnormal urinalysis
13. Amylase/lipase, increased
14. Glucose, increased/decreased
15. Pancreatic/intestinal function tests
16. Calcium (normal/increased); phosphorus
17. Calcium (decreased); phosphorus
18. Assessment of the pituitary-thyroid axis
19. Assessment of the pituitary-adrenocortical axis
20. Evaluation of effusions
21. Evaluation of synovial fluid

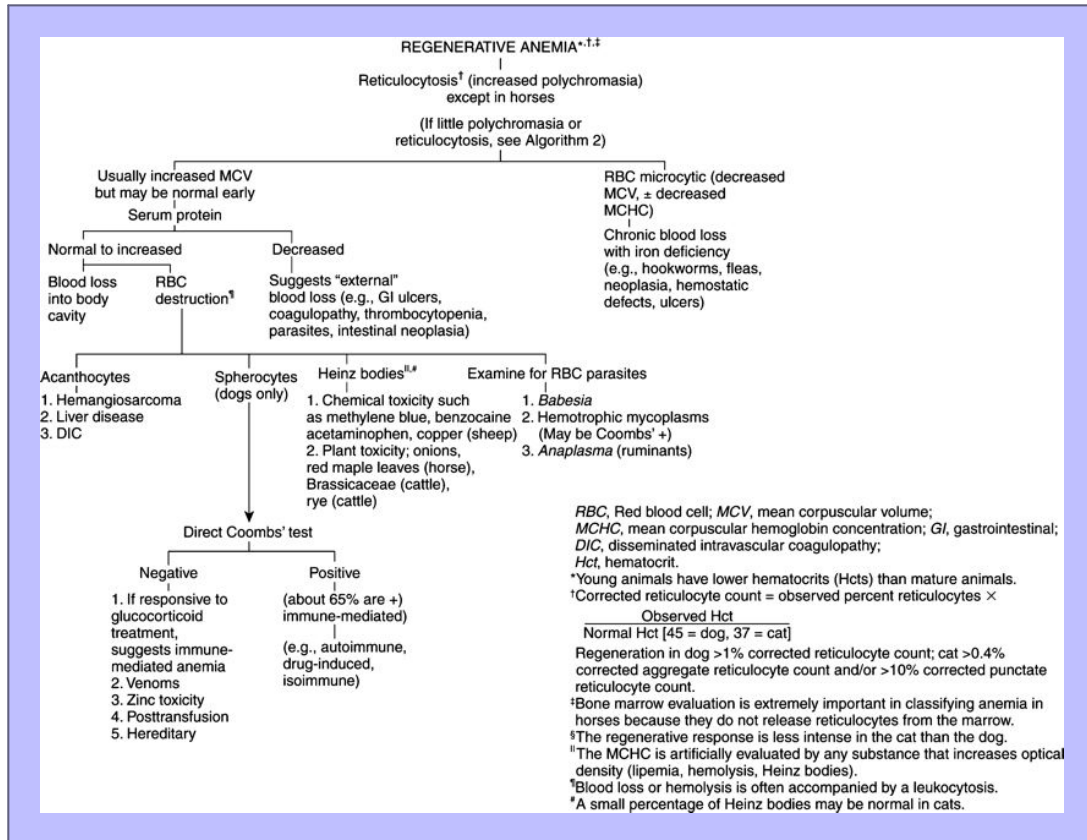
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## 22. Microscopic examination of the cytology specimen

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### 19.1 Algorithm 1

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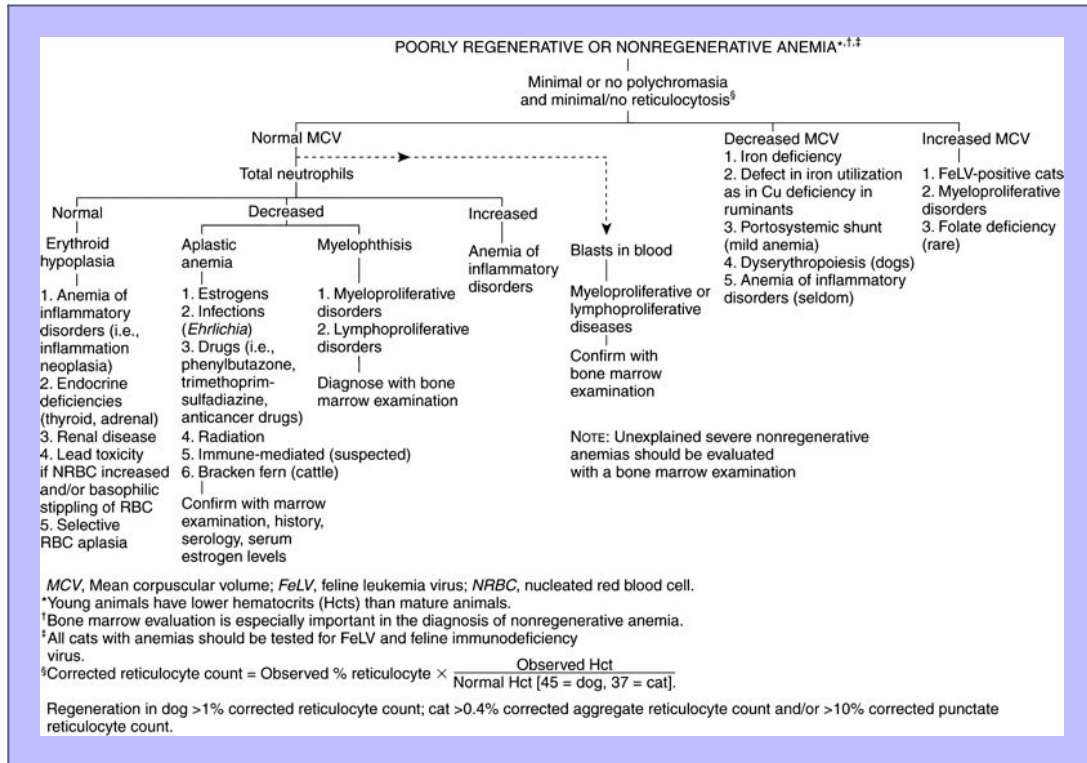


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## 19.2 Algorithm 2

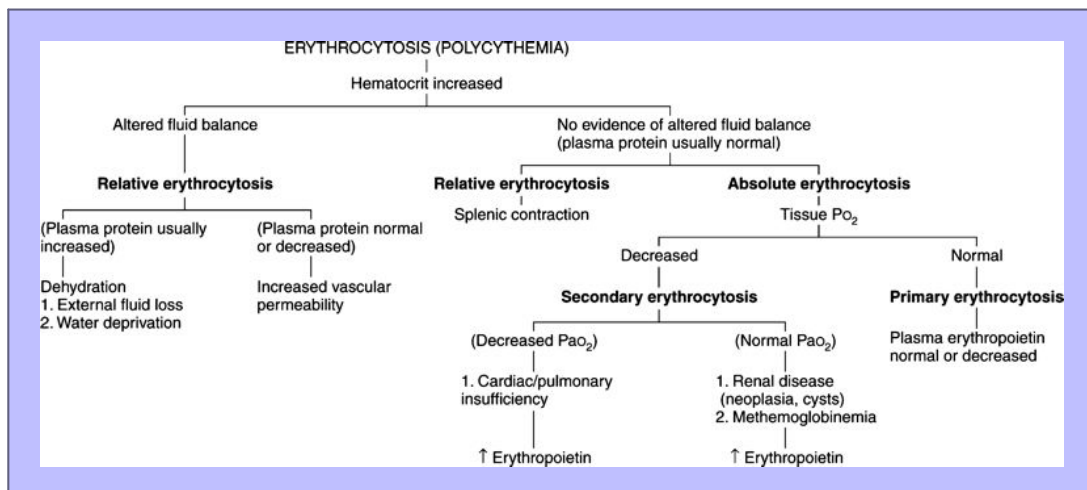
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## 19.3 Algorithm 3

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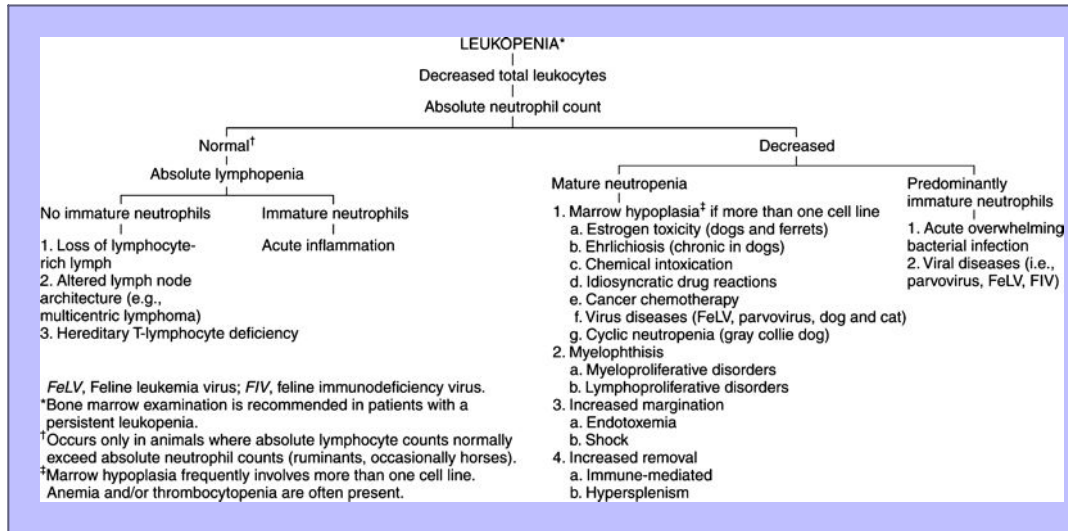


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## 19.4 Algorithm 4

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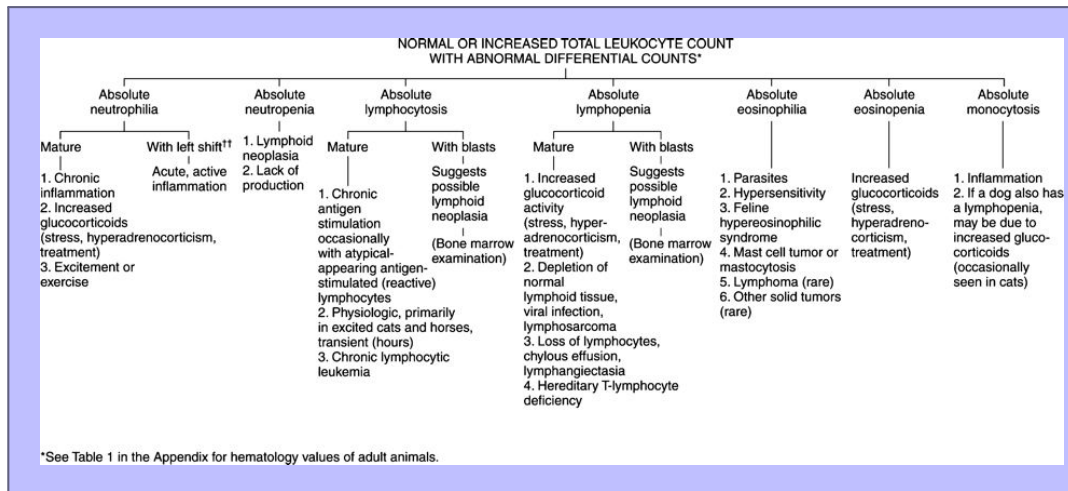


FeLV, Feline leukemia virus; FIV, feline immunodeficiency virus.  
 \*Bone marrow examination is recommended in patients with a persistent leukopenia.  
 †Occurs only in animals where absolute lymphocyte counts normally exceed absolute neutrophil counts (ruminants, occasionally horses).  
 ‡Marrow hypoplasia frequently involves more than one cell line. Anemia and/or thrombocytopenia are often present.

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## 19.5 Algorithm 5

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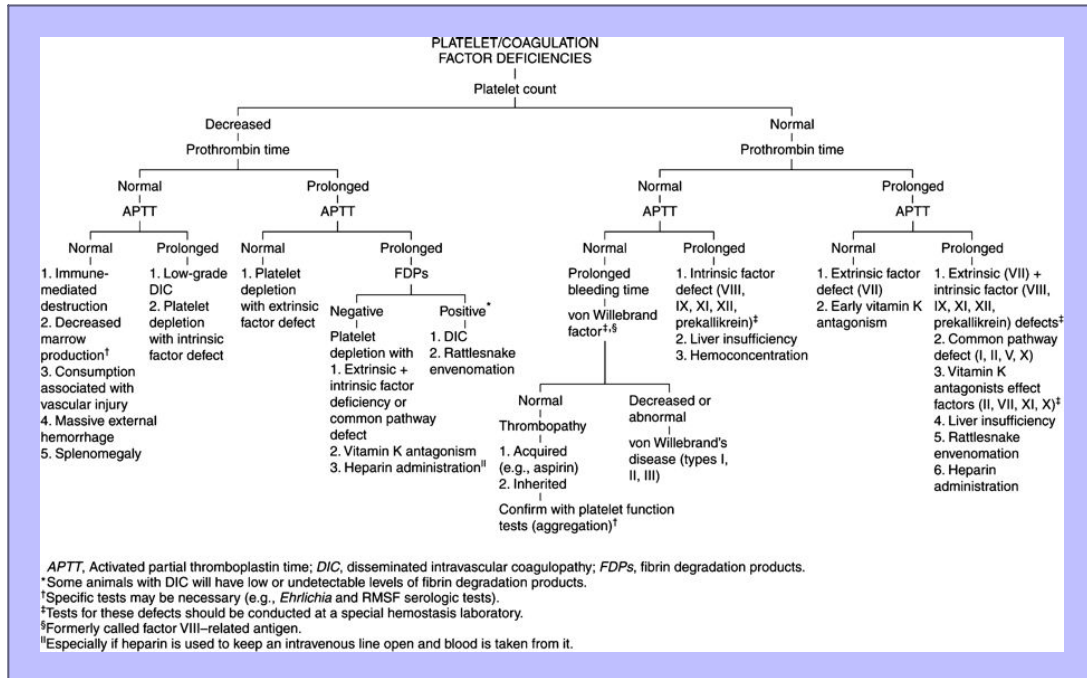
\*See Table 1 in the Appendix for hematology values of adult animals.

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## 19.6 Algorithm 6

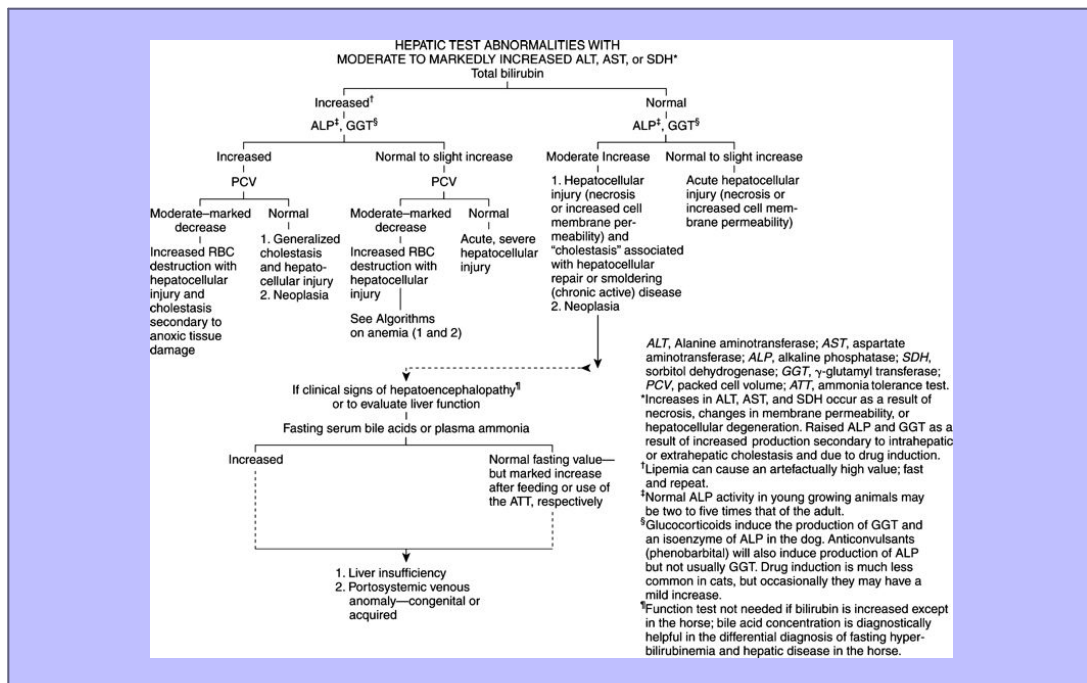
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## 19.7 Algorithm 7

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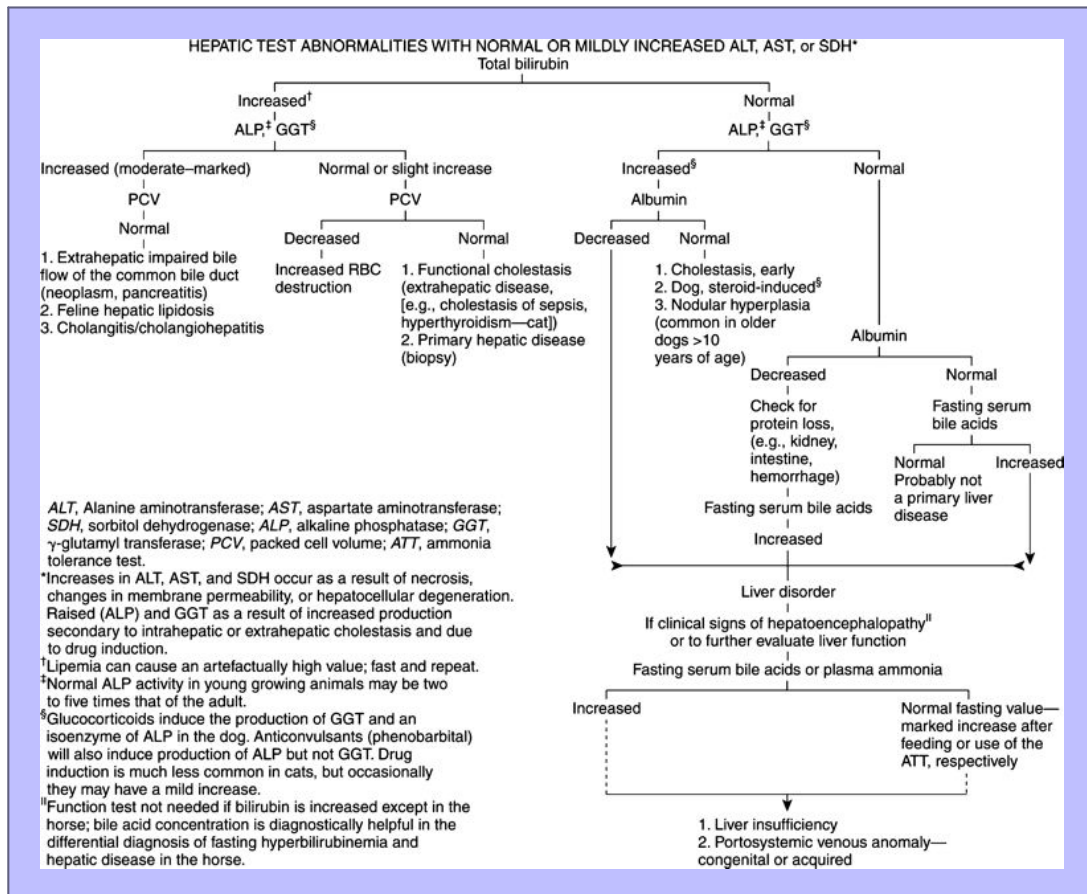


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## 19.8 Algorithm 8

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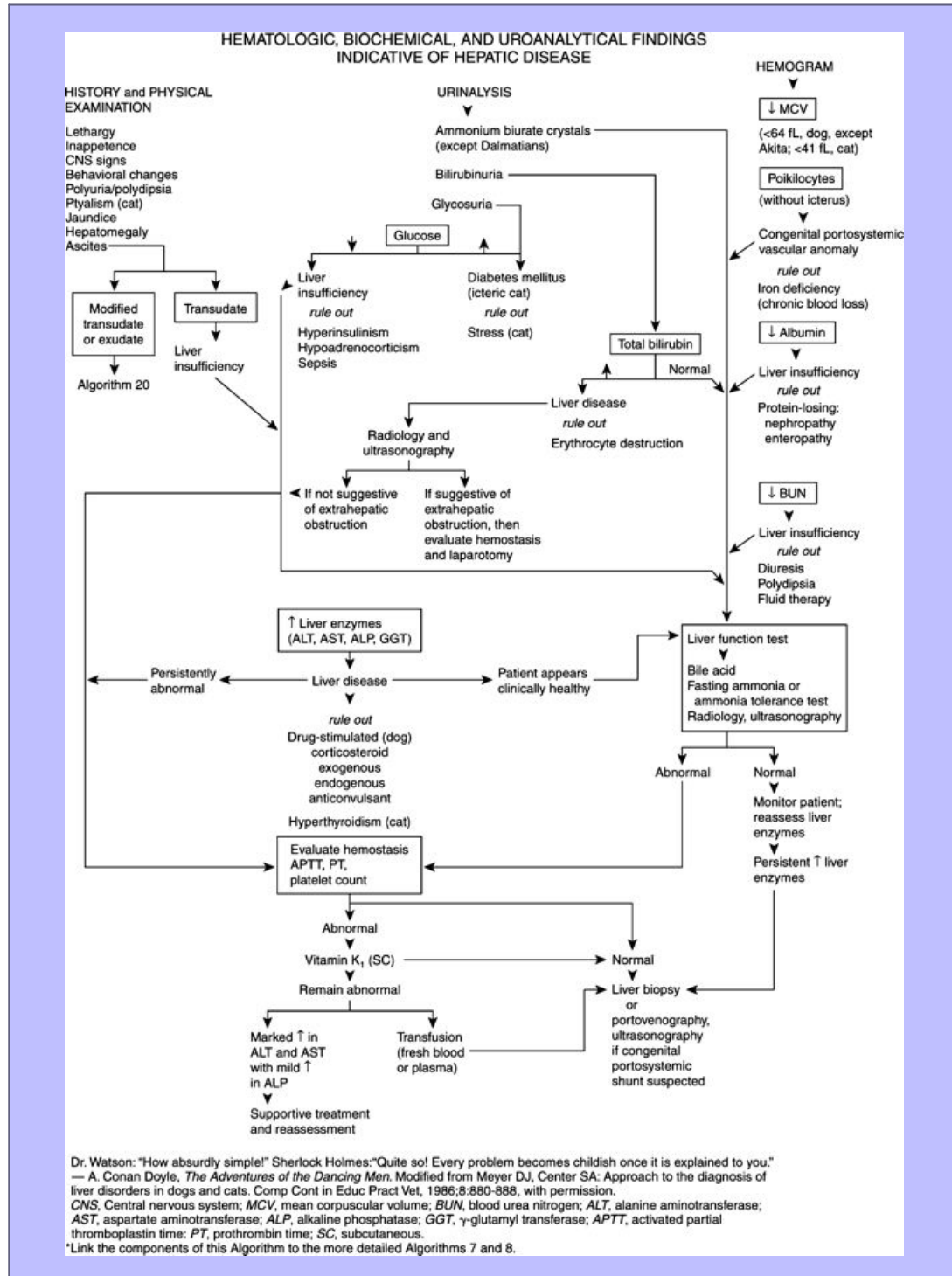


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## 19.9 Algorithm 9

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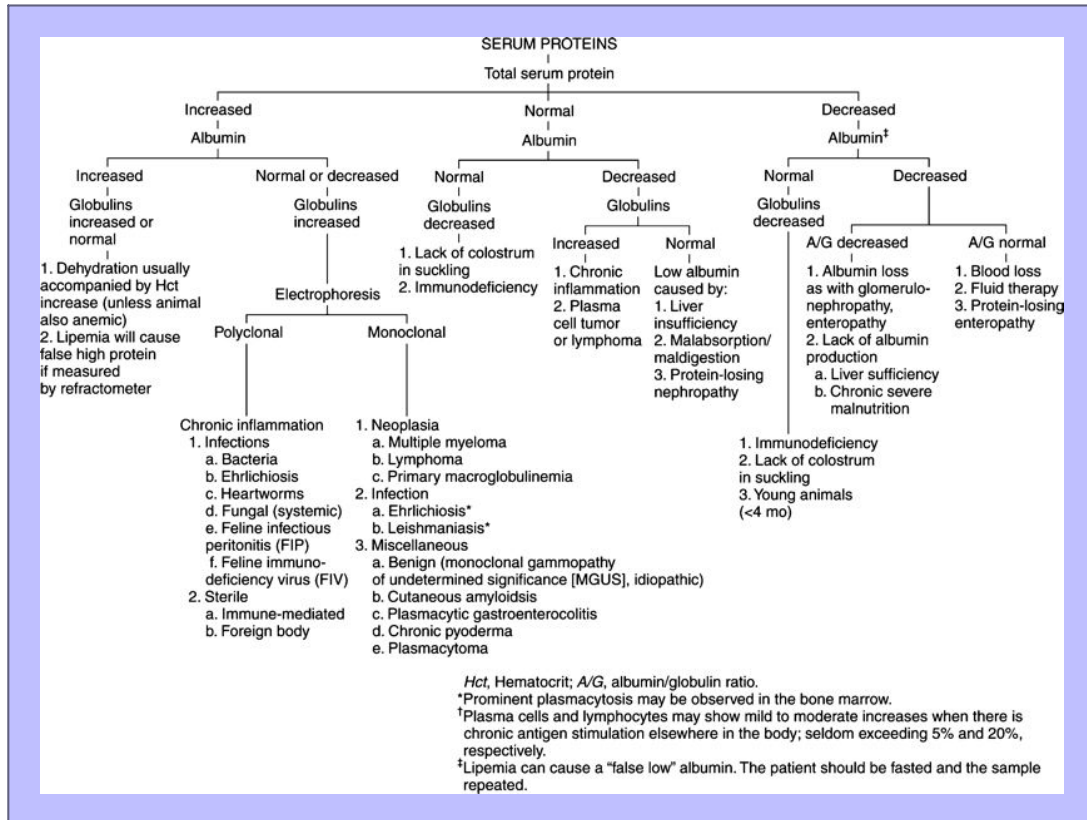


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## 19.10 Algorithm 10

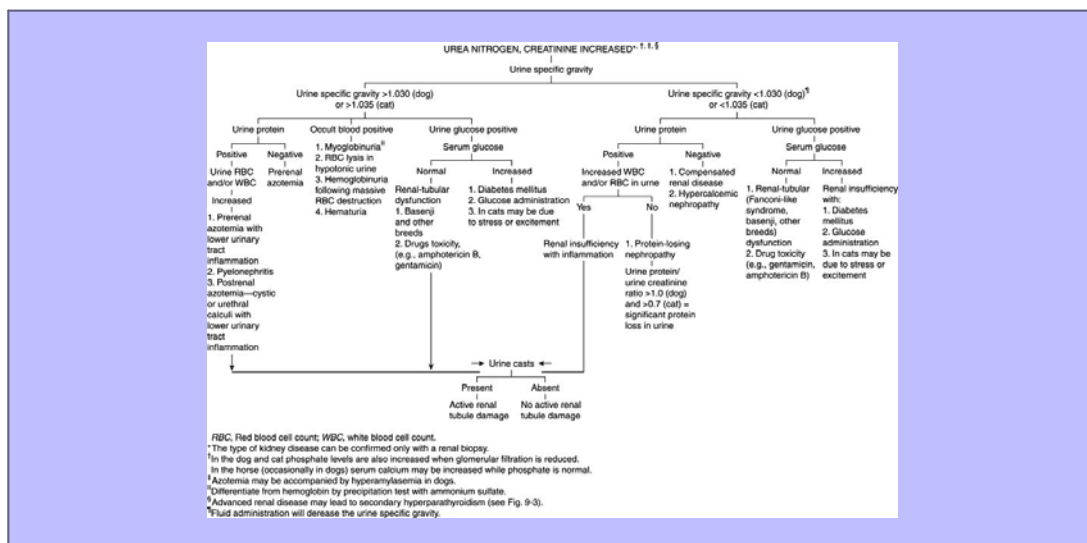
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## 19.11 Algorithm 11

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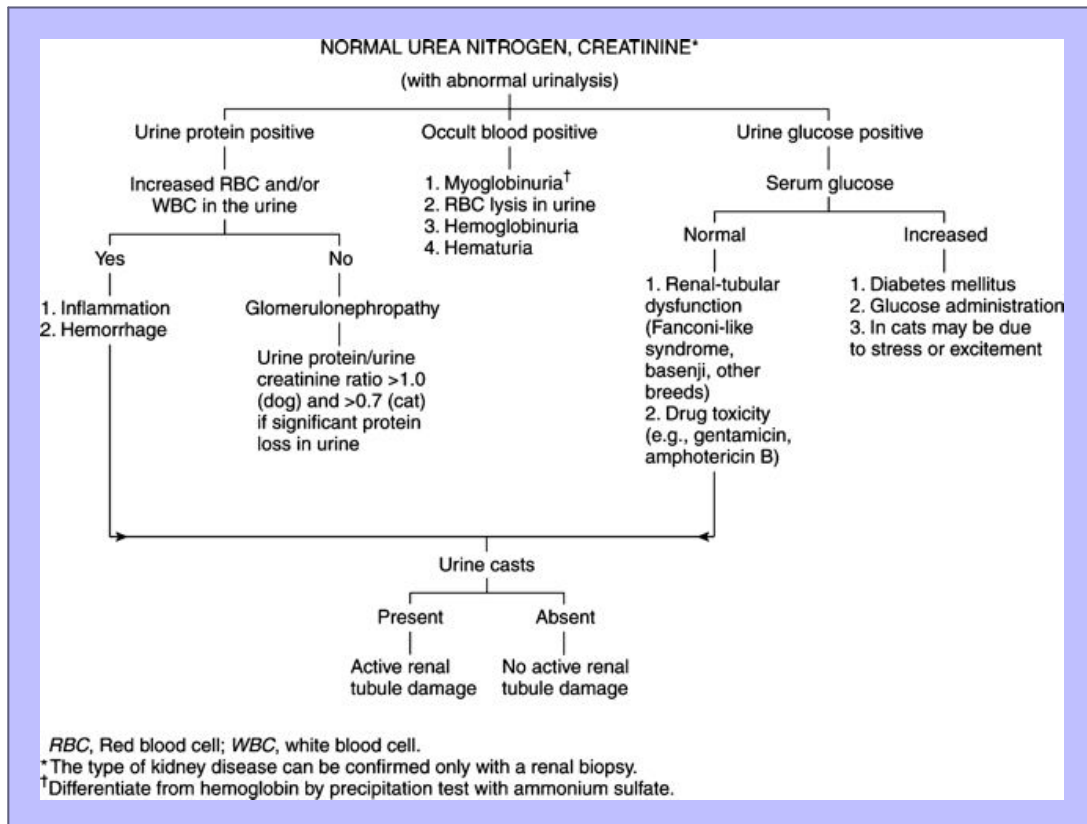


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19.12 Algorithm 12

294

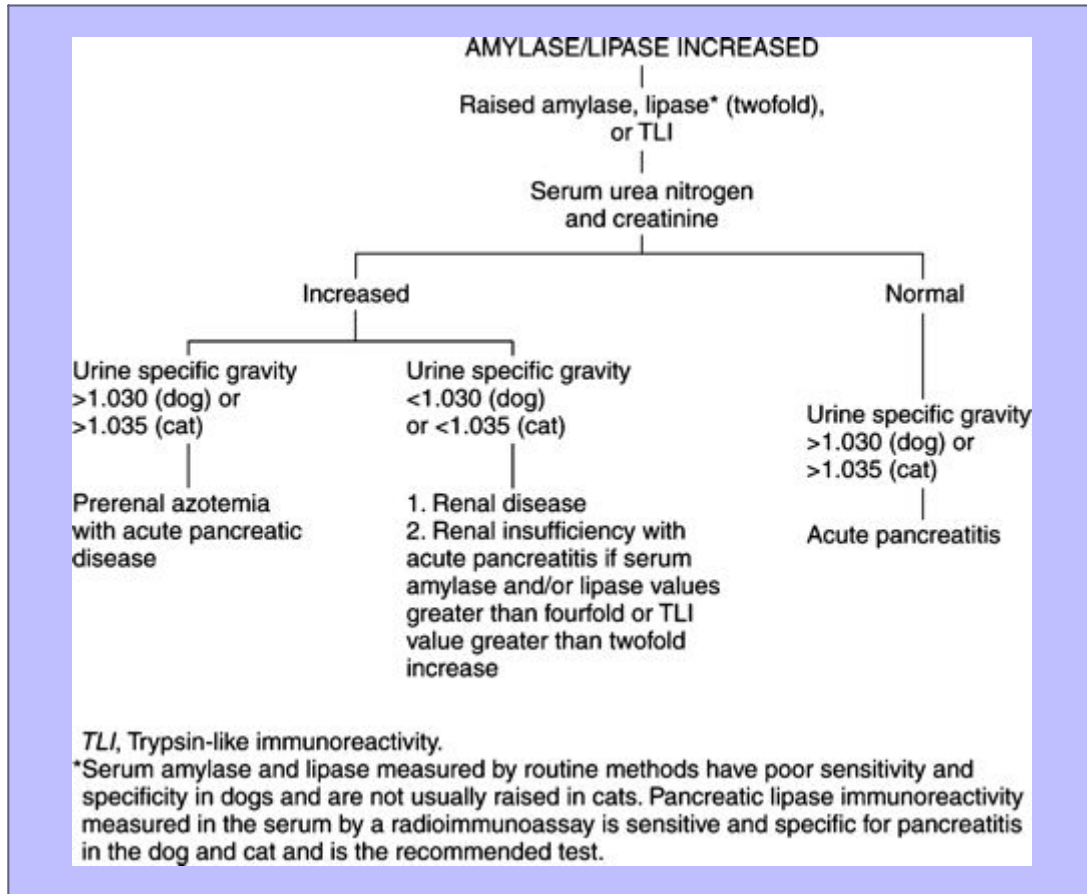


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19.13 Algorithm 13

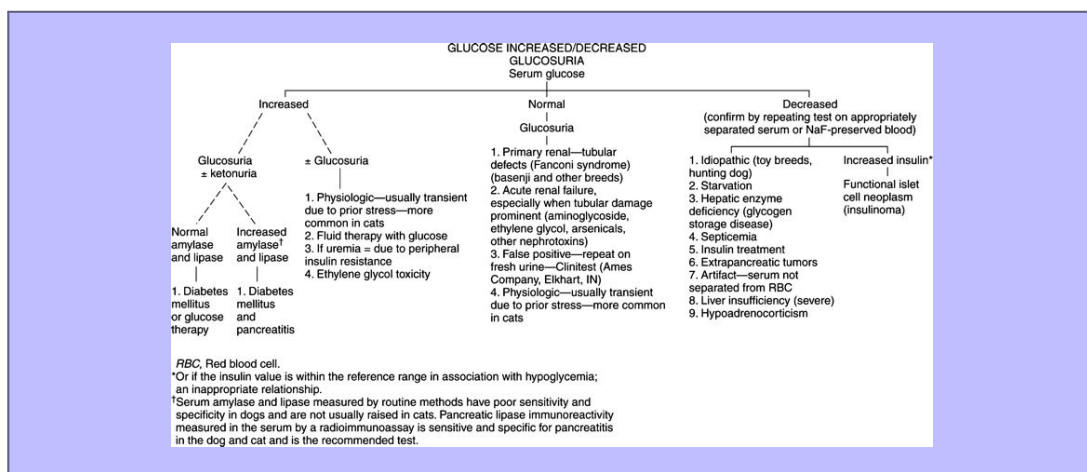
295



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19.14 Algorithm 14

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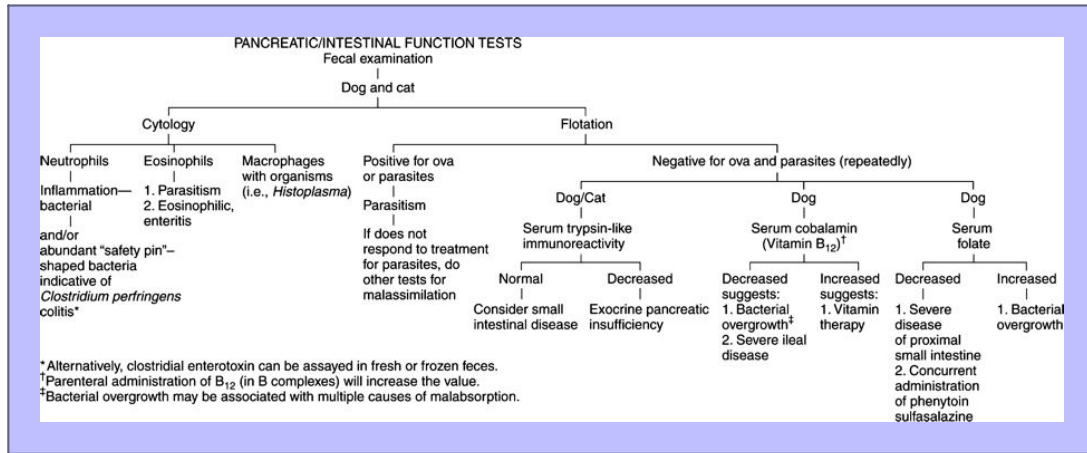


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## 19.15 Algorithm 15

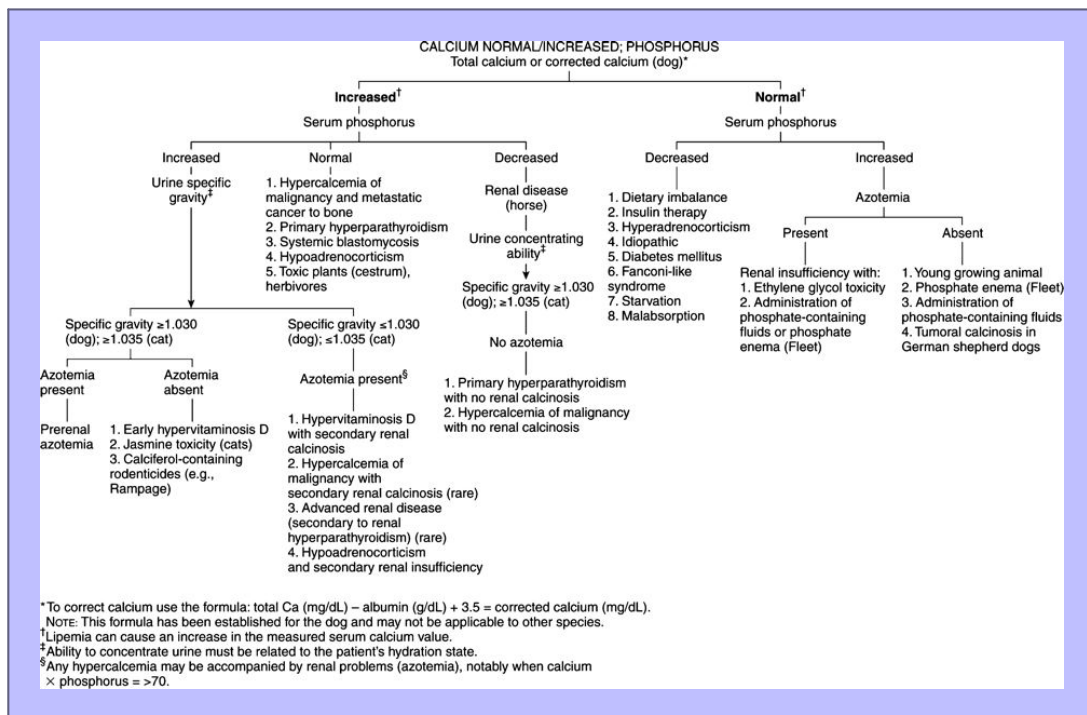
297



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## 19.16 Algorithm 16

298

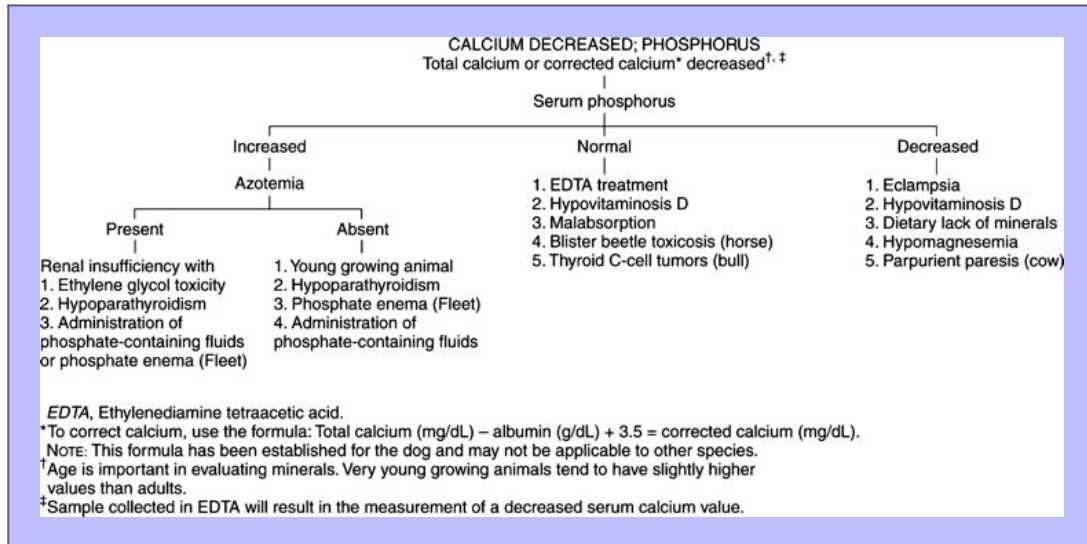


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## 19.17 Algorithm 17

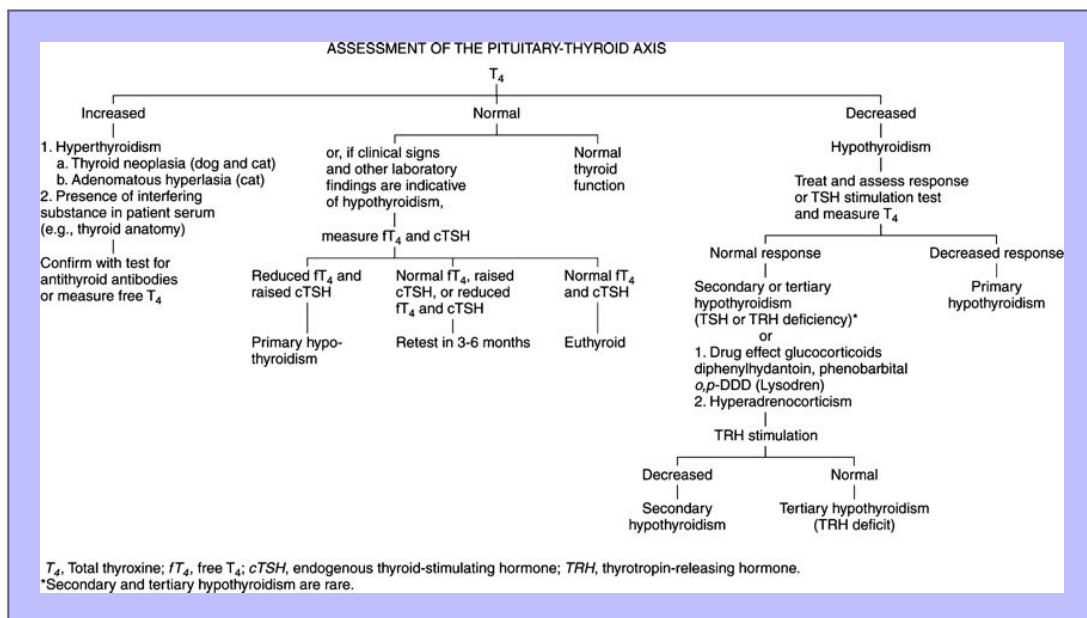
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## 19.18 Algorithm 18

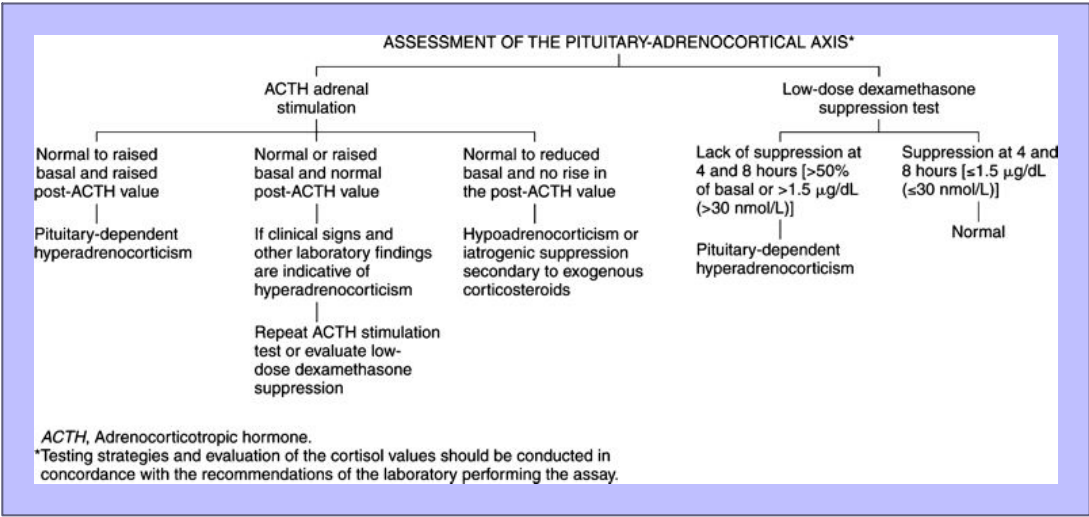
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19.19 Algorithm 19

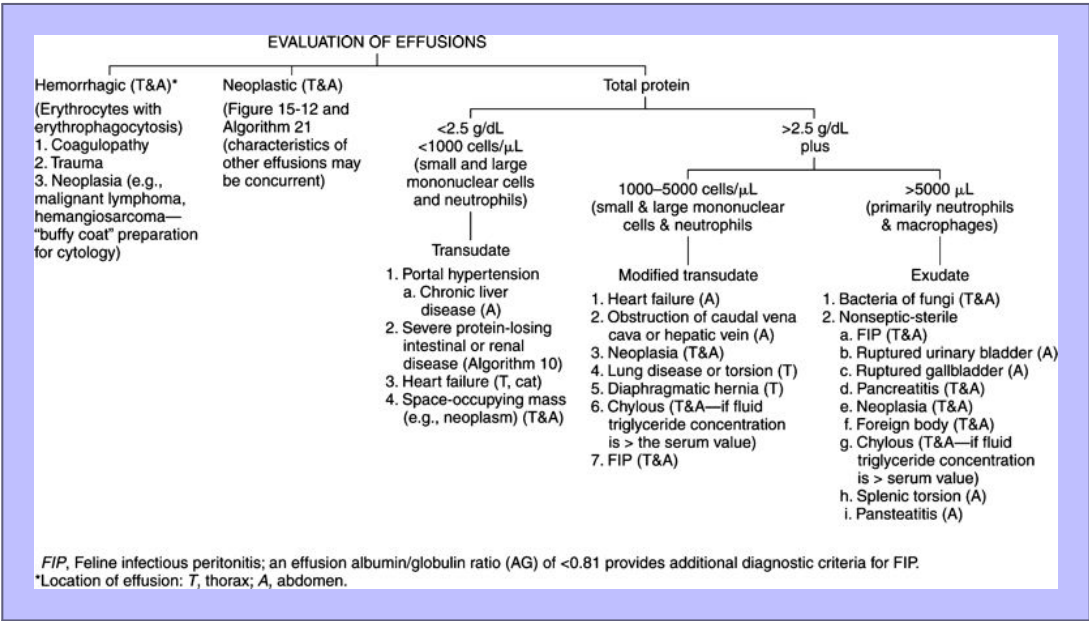
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19.20 Algorithm 20

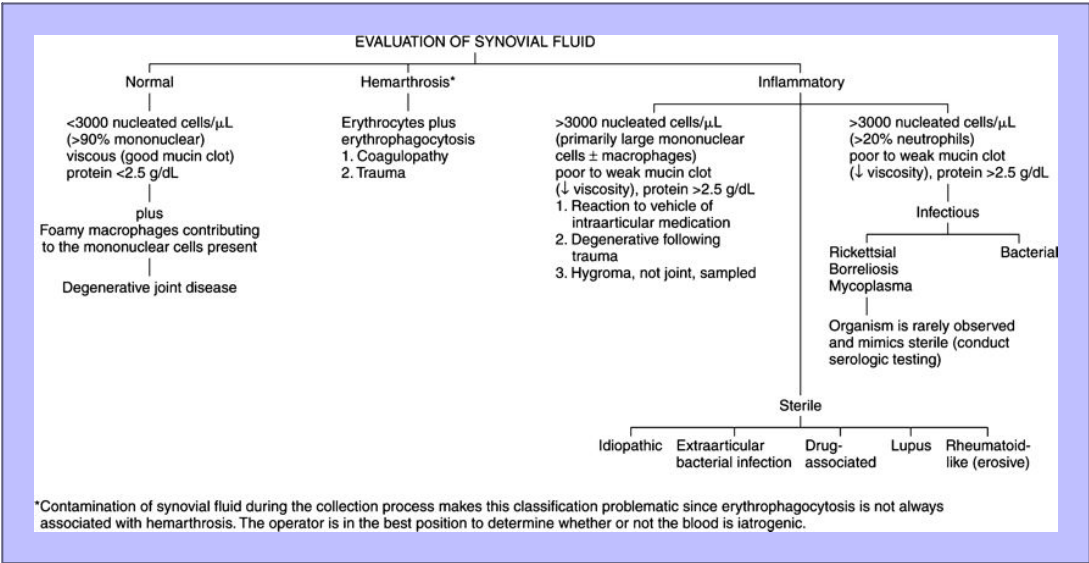
302



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19.21 Algorithm 21

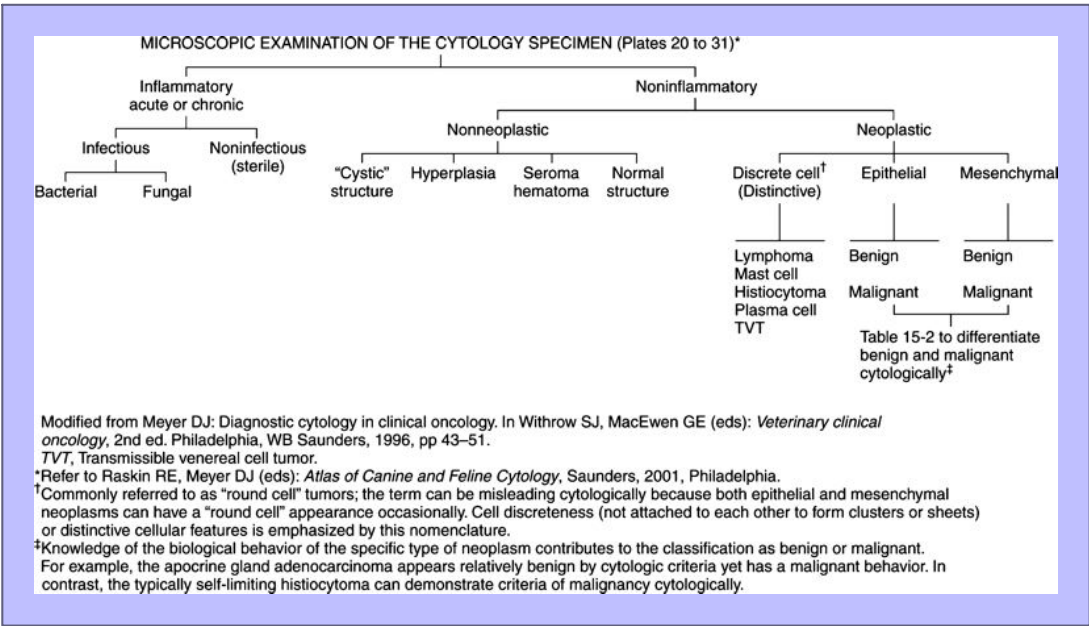
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19.22 Algorithm 22

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## <sup>20</sup>Appendix: Reference Intervals and Conversion Tables

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1. Reference Intervals for Hematology Values for Adult Animals
2. Reference Intervals for Serum Chemistry Values for Adult Animals
3. Conversion of Conventional Units to SI Units in Serum Chemistry
4. Reference Intervals for Hematology Values for Adult Animals Expressed in SI Units
5. Reference Intervals for Serum Chemistry Values for Adult Animals Expressed in SI Units
6. Age-Related Changes in Serum Chemistry Analytes in Beagle Dogs During First Year of Life
7. Variations in Reference Intervals for Serum Chemistry Values by Age: Beagle Dogs, 3 to 14 Years Old
8. Variations in Reference Intervals for Hematology Values by Age: Beagle Dogs, 3 to 14 Years Old
9. Erythrocyte Changes During Pregnancy in Beagles, Brittany Spaniels, and Labrador Retrievers
10. Hematology Values for Growing Healthy Beagle Dogs from Birth to 8 Weeks of Age
11. Hematology Values for Growing Healthy Kittens from Birth to 17 Weeks of Age
12. Normal Values for Biochemical Indicators of Hepatobiliary Disorders in Young Dogs and Cats
13. Age-Related Changes in Plasma and Urine Values in Young Cats
14. Erythrograms of Foals Up to 1 Year of Age
15. Hemostasis Values for Healthy Horses
16. Leukograms of Foals Up to 1 Year of Age
17. Percent Fractional Urinary Excretion of Electrolytes in Cows
18. Normal Percent Fractional Excretion of Electrolytes in the Urine of Domestic Animals

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Table 1 Reference intervals for hematology values for adult animals\*

Test	Units	Canine	Feline	Equine	Bovine	Porcine	Ovine
RBC	$\times 10^6/\mu\text{L}$	5.4–7.8	5.8–10.7	6.4–10.0	5.0–10.0	5.0–8.0	8.0–15.0
Hemoglobin	g/dL	13–19	9–15	11–17	8–15	10–18	8–16
HCT	%	37–54	30–47	32–47	24–46	33–50	24–49
MCV	fL	62–74	41–51	43–54	37–51	50–67	23–48
MCHC	g/dL	32–36	31–35	34–37	33–37	30–34	31–34
MCH	Pg	22–27	13–18	15–19	13–18	17–21	8–12
RDW	%	12–15	14–19	18–22		16–24	
Platelets	$\times 10^5/\mu\text{L}$	1.6–4.3	3–8	1–2.7	2–7.3	2–8	3–8
MPV	fL	6.7–11.1	ND	4.6–7.3		4.5–6.7	
Fibrinogen	mg/dL	100–400	100–300	100–500	200–700	100–500	100–500
Icterus index	units	<5	<5	5–25	0.20	<5	<5
Plasma protein	g/dL	6.0–7.8	6.2–8.0	6.1–8.0	7.0–8.5	6.0–8.0	6.0–7.5
Reticulocytes	$\times 10^3/\mu\text{L}$	<80	<30 agg <500 punc	0	0	<70	0
WBC	$\times 10^3/\mu\text{L}$	6.0–17.0	5.5–19.5	5.2–13.9	4.0–12.0	10.0–22.0	4.0–12.0
Bands	$\times 10^3/\mu\text{L}$	0–0.3	0–0.3	0–0.1	0–0.12		
Neutrophils	$\times 10^3/\mu\text{L}$	3.0–11.5	2.5–12.5	2.2–7.4	0.6–4.0	3.2–10.0	1.0–5.0
Lymphocytes	$\times 10^3/\mu\text{L}$	1.0–4.8	1.5–7.0	1.1–5.3	2.5–7.5	4.4–13.5	2.0–9.0
Monocytes	$\times 10^3/\mu\text{L}$	0.15–1.35	0–0.85	0–0.9	0.03–0.8	0.2–2.2	0–0.75
Eosinophils	$\times 10^3/\mu\text{L}$	0.1–1.25	0–1.5	0–0.6	0–2.4	0.2–2.0	0.1–0.75
Basophils	$\times 10^3/\mu\text{L}$	<0.1	<0.1	<0.3	<0.2	Rare	Rare

From the Veterinary Teaching Hospital-University of Florida.

RBC, Red blood cell count; HCT, hematocrit; MCV, mean corpuscular volume; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin; RDW, red blood cell distribution width; WBC, white blood cell count; MPV, mean platelet volume; agg, aggregate reticulocytes; punc, punctate reticulocytes.

\* Platelet counts determined electronically for all species except the cat. Reference intervals for canine platelet counts determined by manual method is  $2\text{--}5 \times 10^5/\mu\text{L}$ .

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Table 2 Reference intervals for serum chemistry for adult animals

Test	Units	Canine	Feline	Equine	Bovine	Porcine	Ovine
Ammonia	mol/L	0–40	0–40	0–40			
ALP	U/L	10–73	15–92	102–257	29–99	26–362	68–387
ALT	U/L	15–58	30–100	4–12	17–37	32–84	60–84
AST	U/L	16–43	12–56	152–294	48–100	9–113	98–278
Amylase	U/L	510–1864	365–948	9–34	12–107		
Anion gap	mEq/L	11–26	13–24	7–16	12–22		
Bile acid–fast	μmol/L	<5	<2	<15	See text		
Postprandial	μmol/L	<15	<15				
Bilirubin (total)	mg/dL	0.1–0.3	0.1–0.2	0.5–2.1	0.1–0.3	0.1–0.2	0.1–0.4
Calcium	mg/dL	9.0–10.8	7.4–10.5	10.6–13.0	7.9–10.0	8–12	10.4–13
CO <sub>2</sub>	mEq/L	20–27	15–25	26–35	24–34	18–26	21–28
Chloride	mEq/L	110–118	116–125	97–104	94–104	100–105	98–115
Cholesterol	mg/dL	108–266	38–186	50–143	87–254	36–54	50–140
Cholinesterase	U/L	1347–2269	1000–2000				
Cortisol (basal)	μg/dL	1.0–6.8	0.3–2.6				
CK (CPK)	U/L	40–254	59–527	113–333	44–228		
Creatinine	mg/dL	0.5–1.4	0.7–1.8	1.0–1.9	0.7–1.1	1.0–2.7	1.2–1.9
Folate	μg/L	7.5–17.5	13.4–38				
GGT	U/L	1–5	0–2	9–25	20–48		
Glucose	mg/dL	77–120	58–120	76–127	37–71	65–95	50–80
Iron	μg/dL	84–233	65–233	74–209	57–162	91–199	166–222
Lipase	U/L	13–200	0–83				
Magnesium	mEq/L	1.2–2.0	1.5–3.5	1.3–2.0	1.4–2.3		
Osmolality	mOsm/kg	291–315	292–356	282–302			
Phosphorus, inorganic	mg/dL	2.4–6.1	2.6–7.9	2.0–4.3	4.6–9.0	5.3–9.6	5.0–7.3
Potassium	mEq/L	4.2–5.6	4.0–5.3	2.4–5.2	4.0–5.3	4.9–7.0	4.0–6.0
Protein (total)	g/dL	5.4–7.1	5.7–7.9	5.5–7.3	5.9–7.7	7.0–8.9	6.0–7.9
Albumin	g/dL	2.5–3.6	2.3–3.4	2.7–4.2	2.7–4.3	1.9–3.3	2.4–3.9
Globulin	g/dL	2.4–4.0	2.6–4.5	2.1–3.8	2.5–4.1	5.3–6.4	3.5–5.7
Sodium	mEq/L	145–153	151–158	136–142	136–144	139–152	136–154
SDH (SD)	U/L	2.9–8.2	3.9–7.7	1.9–5.8	4.3–15.3	1–6	6–28
T <sub>3</sub>	ng/dL	85–250	85–250				
T <sub>4</sub>	μg/dL	1.2–3.0	1.2–3.0				
T <sub>4</sub> (free)	ng/dL	0.7–3.3					
TLI	μg/dL	5–35					
Triglycerides	mg/dL	20–112	10–114	4–44	0–14		
Urea nitrogen	mg/dL	7–25	18–33	12–26	10–26	8–24	18–31

From the Veterinary Teaching Hospital-University of Florida, with permission.

ALP, Alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatinine kinase; CPK, creatine phosphokinase; GGT, γ-glutamyl transferase; SDH (SD), sorbitol dehydrogenase; T<sub>3</sub>, triiodothyronine; T<sub>4</sub>, thyroxine; TLI, trypsin-like immunoreactivity.

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Table 3 Conversion of conventional units to SI units in serum chemistry

Component	Conventional Unit	Factor	SI Units
ACTH	pg/mL	0.22	pmol/L
Albumin	g/dL	10	g/L
Aminotransferases (ALT, AST)	U/L	1	U/L
Ammonia	μg/dL	0.5872	mol/L
Amylase	Somogyi units	1.85	U/L
Bicarbonate	mEq/L	1	mmol/L
Bilirubin	mg/dL	17.1	mol/L
Calcium	mg/dL	0.25	mmol/L
CO <sub>2</sub>	mEq/L	1	mmol/L
Chloride	mEq/L	1	mmol/L
Cholinesterase	U/L	1	U/L
Cholesterol	mg/dL	0.026	mmol/L
Cortisol	μg/dL	27.6	nmol/L
Creatine kinase (CK)	U/L	1	U/L
Creatinine	mg/dL	88.4	mol/L
Creatinine clearance	mL/min	0.0167	mL/sec
Fibrinogen	mg/dL	0.01	g/L
γ-Glutamyl transferase (GGT, γ-glutamyl-transpeptidase [GGTP])	U/L	1	U/L
Globulin	g/dL	10	g/L
Glucose	mg/dL	0.055	mmol/L
Insulin	μU/L	7.175	pmol/L
Iron-binding capacity	μg/dL	0.179	mol/L
Iron (total)	μg/dL	0.179	mol/kg
Lipase	U/L	1	U/L
Magnesium	mEq/L	0.5	mmol/L
Magnesium	mg/dL	0.41	mmol/L
Osmolality	mOsm/kg	1	mmol/L
Phosphatase, alkaline (ALP)	U/L	1	U/L
Phosphorus, inorganic	mg/dL	0.323	mmol/L
Potassium	mEq/L	1	mmol/L
Protein (total)	g/dL	10	g/L
Sodium	mEq/L	1	mmol/L
T <sub>4</sub>	μg/dL	12.87	nmol/L
fT <sub>4</sub>	ng/dL	12.87	pmol/L
T <sub>3</sub>	ng/dL	0.0154	nmol/L
Triglycerides	mg/dL	0.011	mol/L
Urea nitrogen (BUN)	mg/dL	0.357	mmol/L
Uric acid	mg/dL	0.059	mmol/L
Xylose	mg/dL	0.0666	mmol/L
Zinc	μg/dL	0.1530	mol/L

ACTH, Adrenocorticotrophic hormone; ALT, alanine aminotransferase; AST, aspartate aminotransferase; T<sub>4</sub>, thyroxine; fT<sub>4</sub>, free thyroxine; T<sub>3</sub>, triiodothyronine; BUN, blood urea nitrogen.

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# Veterinary Laboratory Medicine: Interpretation & Diagnosis, 3rd Edition

Table 4 Reference intervals for hematology values for adult animals expressed in SI units

Test	Units	Canine	Feline	Equine	Bovine	Porcine	Ovine
RBC	$\times 10^{12}/L$	5.4–7.8	5.8–10.7	6.4–10.0	5.0–10.0	5.0–8.0	8.0–15.0
Hemoglobin	g/L	130–190	90–150	110–170	80–150	100–180	80–160
HCT	Volume fraction	0.37–0.54	0.30–0.47	0.32–0.47	0.24–0.46	0.33–0.50	0.24–0.49
MCV	fL	64–74	41–51	43–54	37–51	50–67	23–48
MCHC	g/L	340–360	310–350	340–370	330–370	300–340	310–340
MCH	pg	22–27	13–18	15–19	13–18	17–21	8–12
RDW	%	12–15	14–19	18–22	16–24		
Platelets	$\times 10^9/L$	160–430	300–800	100–270	200–730	200–800	300–800
MPV	fL	6.7–11.1	ND	4.6–7.3	4.5–6.7		
Fibrinogen	g/L	1–4	1–3	1–5	2–7	1–5	1–5
Icterus index	units	<5	<5	5–25	0–20	<5	<5
Plasma protein	g/L	60–78	62–80	61–80	70–85	60–80	60–75
Reticulocytes	$\times 10^9/L$	<80	<30 agg <500 punc	0	0	<70	0
WBC	$\times 10^9/L$	6.0–17.0	5.5–19.5	5.2–13.9	4.0–12.0	10–22	4.0–12.0
Bands	$\times 10^9/L$	0–0.3	0–0.3	0–0.1	0–0.12		
Neutrophils	$\times 10^9/L$	3.0–11.5	2.5–12.5	2.2–7.4	0.6–4.0	3.2–10.0	1.0–5.0
Lymphocytes	$\times 10^9/L$	1.0–4.8	1.5–7.0	1.1–5.3	2.5–7.5	4.4–13.5	2.0–9.0
Monocytes	$\times 10^9/L$	0.15–1.35	0–0.85	0–0.9	0.03–0.8	0.2–2.2	0–0.75
Eosinophils	$\times 10^9/L$	0.1–1.25	0–1.5	0–0.6	0–2.4	0.2–2.0	0.1–0.75
Basophils	$\times 10^9/L$	<0.1	<0.1	<0.3	<0.2	Rare	Rare

RBC, Red blood cell count; HCT, hematocrit; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin; RDW, red blood cell distribution width; MPV, mean platelet volume; WBC, white blood cell count; agg, aggregate reticulocyte; punc, punctate reticulocyte.

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Table 5 Reference intervals for serum chemistry values for adult animals expressed in SI units

Test	Units	Canine	Feline	Equine	Bovine	Porcine	Ovine
Ammonia	μmol/L	0–40	0–40		0–40		
ALP	U/L	10–73	15–92	102–257	29–99	26–362	68–387
ALT	U/L	15–58	30–100	4–12	17–37	32–84	60–84
AST	U/L	16–43	12–56	152–294	48–100	9–113	98–278
Amylase	U/L	510–1864	365–948	9–34	12–107		
Anion gap	mmol/L	11–26	13–24	7–16	12–22		
Bile acid–fast	μmol/L	<5	<2	<15	See text		
Postprandial	μmol/L	<15	<10				
Bilirubin (total)	μmol/L	1.7–5.1	1.7–3.4	9–36	1.7–5.1	1.7–3.4	1.7–7
Calcium	mmol/L	2.25–2.7	1.85–2.6	2.65–3.25	1.98–2.5	2–3	2.6–3.25
CO <sub>2</sub>	mmol/L	20–27	15–25	26–35	24–34	18–26	21–28
Chloride	mmol/L	110–118	116–125	97–104	94–104	100–105	98–115
Cholesterol	mmol/L	2.8–6.9	1.0–4.8	1.3–3.7	2.3–6.6	1.0–1.4	1.3–3.6
Cholinesterase	U/L	1347–2269	1000–2000				
Cortisol (basal)	mmol/L	28–188	5–72				
CK (CPK)	U/L	40–254	59–527	113–333	44–228		
Creatinine	μmol/L	44–124	62–159	88–168	62–97	88–239	106–168
GGT	U/L	1–5	0–2	9–25	20–48		
Glucose	mmol/L	4.3–6.7	3.2–6.7	4.2–7.0	2.1–3.9	3.6–5.2	2.8–4.4
Iron	μmol/L	15–42	12–42	13–37	10–29	16–36	30–40
Lipase	U/L	13–200	0–83				
Magnesium	mmol/L	0.6–1.0	0.7–1.7	0.6–1.0	0.7–1.1		
Osmolality	mmol/kg	291–315	292–356	282–302			
Phosphorus, inorganic	mmol/L	0.8–2	0.8–2.6	0.7–1.4	1.5–2.9	1.7–3	1.6–2.4
Potassium	mmol/L	4.2–5.6	4.0–5.3	2.4–5.2	4.0–5.3	4.9–7.1	4.0–6.0
Protein (total)	g/L	54–71	57–79	55–73	59–77	70–89	60–79
Albumin	g/L	25–36	23–34	27–42	27–43	19–33	24–39
Globulin	g/L	24–40	26–45	21–38	25–41	53–64	35–57
Sodium	mmol/L	145–153	151–158	136–142	136–144	139–152	136–154
SDH (SD)	U/L	3–8	4–8	2–6	4–15	1–6	6–28
T <sub>3</sub>	nmol/L	0.9–1.3	0.7–1.2	0.9–1.2			
T <sub>4</sub>	μmol/L	1.6–2.4	1.2–3.0	1.4–1.7			
fT <sub>4</sub>	pmol/L	1.2–2.4	1.8–2.7	0.7–0.9			
Triglycerides	mmol/L	0.2–1.3	0.1–1.3	0.1–0.5	0–0.2		
Urea nitrogen	mmol/L	2.5–8.9	6.4–11.8	4.3–9.3	3.6–9.3	2.86–8.6	6.4–11

ALP, Alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatinine kinase; CPK, creatine phosphokinase; GGT, γ-glutamyl transferase; SDH (SD), sorbitol dehydrogenase; T<sub>3</sub>, triiodothyronine; T<sub>4</sub>, thyroxine; fT<sub>4</sub>, free thyroxine.

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Table 6 Age-related changes in serum chemistry analytes in beagle dogs during first year of life

Age	2 weeks		4 weeks		6 weeks		8 weeks		3 months		6 months		9 months		12 months	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
<b>Analytes That Change Rapidly During First 12 Weeks</b>																
Urea N	24.7	24.1	20.1	15.9	14.6	15.5	14.0	15.1	11.5	10.3						
(mg/dL)	(9.8)	(6.3)	(3.6)	(3.5)	(2.9)	(2.2)	(5.0)	(4.0)	(2.6)	(2.4)						
Cholesterol	247.0	233.3	268.5	271.9	196.4	159.5	145.6	125.2	146.5	146.5						
(mg/dL)	(37.9)	(37.9)	(8.5)	(53.1)	(49.6)	(47.5)	(37.2)	(28.3)	(20.6)	(19.9)						
Triglyceride	98.0	97.8	100.2	73.3	65.2	54.3	63.0	68.7	31.4	33.4						
(mg/dL)	(42.0)	(59.4)	(31.4)	(30.7)	(31.1)	(19.8)	(17.1)	(19.2)	(5.3)	(8.2)						
LDH	86.1	86.9	106.5	51.9	71.1	45.3	49.4	54.5	35.2	33.6						
(U/L)	(41.7)	(83.0)	(70.4)	(31.4)	(28.1)	(21.5)	(15.6)	(23.6)	(9.5)	(9.8)						
T <sub>4</sub>	9.9	9.7	7.4	8.4	5.5	5.6	4.4	4.1	2.8	2.8						
(µg/dL)	(2.2)	(1.5)	(1.7)	(2.7)	(1.1)	(3.3)	(1.3)	(1.4)	(0.5)	(0.6)						
Glucose	132.8	132.2	134.5	139.5	130.5	125.3	121.8	115.3	108.5	104.5						
(mg/dL)	(13.1)	(12.5)	(12.5)	(17.6)	(11.9)	(14.3)	(6.3)	(10.7)	(7.9)	(11.0)						
GGT	10.8	6.8	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0						
(U/L)	(9.6)	(8.6)	(0.0)	(0.6)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)						
Bilirubin	0.5	0.8	0.5	0.3	0.4	0.4	0.3	0.4	0.3	0.3						
(mg/dL)	(0.3)	(0.6)	(0.2)	(0.2)	(0.2)	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)						
ALT	9.0	7.3	12.1	9.9	18.2	16.8	22.5	21.3	21.9	23.9						
(U/L)	(3.8)	(1.8)	(6.0)	(3.3)	(7.8)	(7.9)	(10.3)	(10.7)	(7.6)	(6.9)						
<b>Analytes That Change Gradually During First Year of Life</b>																
ALP	149	127	88	99	103	112	122	125	145	143	93	90	66	64	43	59
(U/L)	(33)	(23)	(24)	(23)	(24)	(28)	(22)	(39)	(20)	(29)	(16)	(17)	(17)	(12)	(13)	(35)
Phosphorus <sub>i</sub>	8.8	8.8	8.1	8.1	8.4	8.5	8.9	9.0	8.0	7.8	7.2	7.0	5.5	5.3	3.9	4.3
(mg/dL)	(0.9)	(1.0)	(1.2)	(0.6)	(0.8)	(0.3)	(0.4)	(0.8)	(0.9)	(0.5)	(0.6)	(0.9)	(0.7)	(0.4)	(0.9)	(0.7)
Calcium	12.8	13.3	13.1	12.4	12.7	12.4	13.5	12.9	11.8	12.0	12.8	12.2	11.7	11.3	10.9	11.0
(mg/dL)	(2.0)	(1.3)	(1.1)	(0.9)	(0.7)	(0.9)	(0.8)	(1.0)	(0.5)	(0.9)	(0.80)	(1.1)	(1.0)	(0.8)	(0.6)	(0.4)
Creatinine	0.5	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.6	0.6	0.9	0.8	0.9	0.9	1.0	0.9
(mg/dL)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
AST	16	16	14	13	18	15	20	18	21	20	29	27	28	26	24	22
(U/L)	(4)	(5)	(3)	(2)	(4)	(4)	(5)	(5)	(3)	(4)	(5)	(5)	(6)	(4)	(3)	(4)
Protein	3.8	4.0	3.8	4.0	4.3	4.2	4.4	4.2	4.9	4.6	5.3	5.4	5.8	5.7	5.9	5.8
(g/dL)	(0.2)	(0.2)	(0.3)	(0.3)	(0.4)	(0.2)	(0.4)	(0.4)	(0.2)	(0.2)	(0.3)	(0.3)	(0.4)	(0.2)	(0.2)	(0.3)

From Wolford ST, Schroer RA, Gohs FX, et al: Effect of age on serum chemistry profile, electrophoresis and thyroid hormones in beagle dogs two weeks to one year of age. Vet Clin Pathol 1988;17:35–42. M, Male; F, female; LDH, lactate dehydrogenase; T<sub>4</sub>, thyroxine; GGT, γ-glutamyl transferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase.

\* Mean values (1 SD).

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**Table 7** Variations in reference intervals for serum chemistry values by age: beagle dogs, 3 to 14 years old

Laboratory test*	3 years	6 years	9 years	12 years	14 years
ALP (U/L)	40–75	39–115	51–140	58–112	44–143
ALT (U/L)	35–61	30–55	27–51	58–141	41–68
AST (U/L)	24–32	21–29	24–40	30–50	25–34
Bilirubin, total (mg/dL)	0–0.1	0.1–0.1	0.05–0.1	0.05–0.1	0.05–0.1
Calcium (mg/dL)	10–11	10–10	9–10	10–11	10–11
Chloride (mEq/L)	109–114	103–110	107–112	105–108	107–111
Cholesterol (mg/dL)	139–171	154–224	126–300	156–248	15–231
Triglycerides (mg/dL)	23–36	24–54	19–87	34–102	26–38
Creatinine (mg/dL)	0.8–1.0	0.6–1.0	0.5–0.7	0.7–0.9	0.5–0.8
GGT (U/L)	0–17	0–2	2–4	0–7	0–5
Glucose (mg/dL)	91–102	89–104	92–104	80–95	83–100
Iron (µg/dL)	137–285	187–276	41–233	119–189	91–192
LDH (U/L)	74–112	58–145	134–261	179–360	91–216
Phosphorus, inorganic (mg/dL)	3.9–5.1	3.2–4.4	3.9–5.0	3.7–5.2	3.9–4.5
Potassium (mEq/L)	4.6–5.0	4.5–5.1	4.6–5.2	5.0–5.5	4.7–5.3
Protein (g/dL)	5.4–5.9	6.0–6.3	5.8–6.6	6.0–6.6	5.8–6.7
Albumin (g/dL)	3.3–3.6	3.1–3.5	3.0–3.5	3.0–3.3	2.7–3.4
Globulin (g/dL)	2.2–2.6	2.5–2.9	2.6–3.3	3.1–3.5	2.9–3.5
Sodium (mEq/L)	150–154	143–149	149–151	145–149	145–149
Urea nitrogen (mg/dL)	19–22	9–16	10–13	10–18	10–19

From Lowseth LA, Gillett NA, Gerlach RF, et al: The effects of aging on hematology and serum chemistry values in the beagle dog. Vet Clin Pathol 1990;19:13–19.

ALP, Alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; LDH, lactate dehydrogenase.

\* Limits represent the 10th and 90th percentile values.

**Table 8** Variations in reference intervals for hematology values by age: beagle dogs, 3 to 14 years old

Laboratory test*	3 years	6 years	9 years	12 years	14 years
RBC ( $\times 10^6/\mu\text{L}$ )	6.6–7.8	6.5–7.6	5.9–7.4	6.1–7.3	5.7–7.1
Hemoglobin (g/dL)	15–18	16–18	14–18	14–17	14–17
PCV (%)	43–50	44–49	38–50	40–49	40–47
Band neutrophils/ $\mu\text{L}$	0	0	0–63	0–68	0–54
Segmented neutrophils/ $\mu\text{L}$	3944–9287	3605–7724	4207–7217	4724–9587	4464–10,255
Lymphocytes/ $\mu\text{L}$	2185–3318	1334–2467	1667–2702	1676–2658	1628–2453
Monocytes/ $\mu\text{L}$	101–769	173–626	149–620	181–521	189–688
Eosinophils/ $\mu\text{L}$	208–1010	217–500	275–711	99–721	201–408
Basophils/ $\mu\text{L}$	0	0–70	0–102	0	0

From Lowseth LA, Gillett NA, Gerlach RF, et al: The effects of aging on hematology and serum chemistry values in the beagle dog. Vet Clin Pathol 1990;19:13–19.

RBC, Red blood cell count; PCV, packed cell volume.

\* Limits represent the 10th and 90th percentile values.

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Table 9 Erythrocyte changes during pregnancy in beagles, Brittany spaniels, and Labrador retrievers

	Gestation Week 1			Gestation Week 8			Lactation Week 8		
	Beagles	Brittany spaniels	Labrador retrievers	Beagles	Brittany spaniels	Labrador retrievers	Beagles	Brittany spaniels	Labrador retrievers
RBC ( $\times 10^6/\mu\text{L}$ )									
Mean	7.2	6.2	7.3	5.1	5.0	5.6	7.0	5.6	6.7
Range	6.2–9.2	5.2–7.2	5.5–9.1	4.1–6.1	4.1–6.0	4.4–6.8	5.8–8.2	4.4–6.8	5.7–7.7
PCV (%)									
Mean	47	43.3	50	34.3	33.0	38.2	46.6	40.8	44.1
Range*	38.4–54.6	38.5–48.1	42.4–57.6	39.2–50.8	25.2–40.8	30.4–46.0	39.2–54.0	32.8–48.8	36.1–52.1
Hemoglobin (g/dL)									
Mean	17.2	15.9	18.1	12.3	11.8	13.9	16.5	14.7	15.9
Range*	15.2–19.2	14.1–17.7	15.3–20.9	10.3–14.3	9.2–14.4	11.7–16.1	13.9–19.1	11.7–17.7	13.1–18.7
MCV (fL)									
Mean	66.6	70.3	69.2	67.3	72.6	67.9	67.3	73.3	65.9
Range*	59.2–76	59.7–80.9	50.6–87.8	60.7–73.6	58.6–86.6	52.9–82.9	60.7–73.6	63.1–83.4	55.8–76.0
MCHC (g/dL)									
Mean	36.8	36.6	36.2	36	36.4	36.8	35.5	36	36.1
Range*	30.6–43	34.4–38.8	34.2–38.2	31.2–40.8	34.6–38.2	31.8–41.4	31.7–39.4	33.0–39.0	32.7–39.5

From Allard RL, Carlos AD, Faltin EC: Canine hematological changes during gestation and lactation. Comp Anim Pract 1989;19:3–6.

RBC, Red blood cell count; PCV, packed cell volume; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration.

\* Range is  $\pm 2$  SD.

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Table 10 Hematology values for growing healthy beagle dogs from birth to 8 weeks of age

Hematology parameter	Birth	1 Week	2 Weeks	3 Weeks	4 Weeks	6 Weeks	8 Weeks
RBC ( $\times 10^6/\mu\text{L}$ )	4.7–5.6 (5.1) *	3.6–5.9 (4.6)	3.4–4.4 (3.9)	3.5–4.3 (3.8)	3.6–4.9 (4.1)	4.3–5.1 (4.7)	4.5–5.9 (4.9)
Hemoglobin (g/dL)	14–17 (15.2)	10.4–17.5 (12.9)	9–11 (10.0)	8.6–11.6 (9.7)	8.5–10.3 (9.5)	8.5–11.3 (10.2)	10.3–12.5 (11.2)
PCV (%)	45–52.5 (47.5)	33–52 (40.5)	29–34 (31.8)	27–37 (31.7)	27–33.5 (29.9)	26.5–35.5 (32.5)	31–39 (34.8)
MCV (fL)	93.0	89.0	81.5	83.0	73.0	69.0	72.0
MCH (pg)	30.0	28.0	25.5	25.0	23.0	22.0	22.5
MCHC (g/dL)	32.0	32.0	31.5	31.0	32.0	31.5	32.0
NRBC/100 WBC	0–13 (2.3)	0–11 (4.0)	0–6 (2.0)	0–9 (1.6)	0–4 (1.2)	0–0	0–1 (0.2)
WBC ( $\times 10^3/\mu\text{L}$ )	6.8–18.4 (12.0)	9–23 (14.1)	8.1–15.1 (11.7)	6.7–15.1 (11.2)	8.5–16.4 (12.9)	12.6–26.7 (16.3)	12.7–17.3 (15)
Band neutrophils ( $\times 10^3/\mu\text{L}$ )	0–1.5 (0.23)	0–4.8 (0.50)	0–1.2 (0.21)	0–0.5 (0.09)	0–0.3 (0.06)	0–0.3 (0.05)	0–0.3 (0.08)
Segmented neutrophils ( $\times 10^3/\mu\text{L}$ )	4.4–15.8 (8.6)	3.8–15.2 (7.4)	3.2–10.4 (5.2)	1.4–9.4 (5.1)	3.7–12.8 (7.2)	4.2–17.6 (9.0)	6.2–11.8 (8.5)
Lymphocytes ( $\times 10^3/\mu\text{L}$ )	0.5–4.2 (1.9)	1.3–9.4 (4.3)	1.5–7.4 (3.8)	2.1–10.1 (5.0)	1.0–8.4 (4.5)	2.8–16.6 (5.7)	3.1–6.9 (5.0)
Monocytes ( $\times 10^3/\mu\text{L}$ )	0.2–2.2 (0.9)	0.3–2.5 (1.1)	0.2–1.4 (0.7)	0.1–1.4 (0.7)	0.3–1.5 (0.8)	0.5–2.7 (1.1)	0.5–2.7 (1.1)
Eosinophils ( $\times 10^3/\mu\text{L}$ )	0–1.3 (0.4)	0.2–2.8 (0.8)	0.08–1.8 (0.6)	0.07–0.9 (0.3)	0–0.7 (0.25)	0.1–1.9 (0.5)	0–1.2 (0.4)
Basophils ( $\times 10^3/\mu\text{L}$ )	0.0	0–0.2 (0.01)	0.0	0.0	0.015 (0.01)	0.0	0.0

From Earl FL, Melvegar BA, Wilson RL: The hemogram and bone marrow profile of normal neonatal and weanling beagle dogs. Lab Anim Sci 1973;23:690.

RBC, Red blood cell count; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; NRBC, nucleated red blood cell; WBC, white blood cell count.

\* Values expressed as range and (median).

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Table 11 Hematology values for growing healthy kittens from birth to 17 weeks of age

Hematology parameter	0–2 Weeks	2–4 Weeks	4–6 Weeks	6–8 Weeks	8–9 Weeks	12–13 Weeks	16–17 Weeks
RBC ( $\times 10^6/\mu\text{L}$ )	5.29 (4.81–5.77)*	4.67 (4.47–4.87)	5.89 (5.43–6.35)	6.57 (6.05–7.09)	6.95 (6.77–7.13)	7.43 (6.97–7.89)	8.14 (7.60–8.68)
Hemoglobin (g/dL)	12.1 (10.9–13.3)	8.7 (8.3–9.1)	8.6 (8.0–9.2)	9.1 (8.5–9.7)	9.8 (9.4–10.2)	10.1 (9.5–10.7)	11.0 (10.2–11.9)
PCV (%)	35.3 (31.9–38.7)	26.5 (24.9–28.1)	27.1 (25.5–28.7)	29.8 (27.2–32.4)	33.3 (31.9–34.7)	33.1 (29.9–36.3)	34.9 (32.7–37.1)
MCV (fL)	67.4 (63.6–71.2)	53.9 (51.5–56.3)	45.6 (43.0–48.2)	45.6 (43.6–47.6)	47.8 (46.0–49.6)	44.5 (40.9–48.1)	43.1 (40.1–46.1)
MCH (pg)	23.0 (21.8–24.2)	18.8 (17.2–20.4)	14.8 (13.7–16.0)	13.9 (13.3–14.5)	14.1 (13.7–14.5)	13.7 (12.9–14.5)	13.5 (12.7–14.3)
MCHC (g/dL)	34.5 (32.9–36.1)	33.0 (31.0–34.0)	31.9 (30.7–33.1)	30.9 (29.9–31.9)	29.5 (28.7–30.3)	31.3 (29.5–32.1)	31.6 (30.0–33.2)
WBC ( $\times 10^3/\mu\text{L}$ )	9.67 (8.53–10.81)	15.31 (12.89–17.73)	17.45 (14.71–20.19)	18.07 (14.19–21.95)	23.68 (19.9–27.46)	23.10 (16.48–29.92)	19.7 (17.46–21.94)
Band neutrophils ( $\times 10^3/\mu\text{L}$ )	0.06 (0.02–0.10)	0.11 (0.03–0.19)	0.20 (0.08–0.32)	0.22 (0.06–0.38)	0.12 (0.0–0.30)	0.15 (0.01–0.27)	0.16 (0.020–0.30)
Neutrophils ( $\times 10^3/\mu\text{L}$ )	5.96 (4.60–7.32)	6.92 (5.38–8.46)	9.57 (6.27–12.87)	6.75 (4.69–8.81)	11.0 (8.18–13.82)	11.0 (7.46–14.54)	9.74 (7.90–11.58)
Lymphocytes ( $\times 10^3/\mu\text{L}$ )	3.73 (2.69–4.77)	6.56 (5.38–7.74)	6.41 (4.87–7.95)	9.59 (6.45–12.73)	10.17 (6.75–13.59)	10.46 (5.24–15.68)	8.7 (6.58–10.82)
Monocytes ( $\times 10^3/\mu\text{L}$ )	0.01 (0.0–0.03)	0.02 (0.0–0.06)	0.0 (0.0–0.06)	0.01 (0.0–0.03)	0.11 (0.0–0.23)	0.0 (0.0–0.06)	0.02 (0.0–0.06)
Eosinophils ( $\times 10^3/\mu\text{L}$ )	0.96 (0.10–1.82)	1.40 (1.08–1.72)	1.47 (0.97–1.97)	1.08 (0.68–1.48)	2.28 (1.66–2.90)	1.55 (0.85–2.25)	1.00 (0.62–1.38)
Basophils ( $\times 10^3/\mu\text{L}$ )	0.02 (0.0–0.04)	0 (0.0–0.06)	0 (0.0–0.06)	0.02 (0.0–0.06)	0 (0.0–0.09)	0.03 (0.0–0.09)	0 (0.0–0.09)

From Meyers-Wallen VN, Haskins ME, Patterson DF: Hematologic values in healthy neonatal, weanling and juvenile kittens. Am J Vet Res 1984;45:1322. Range calculated from mean  $\pm$  2 SDs.  
*RBC*, Red blood cell count; *PCV*, packed cell volume; *MCV*, mean cell volume; *MCH*, mean cell hemoglobin; *MCHC*, mean cell hemoglobin concentration; *WBC*, white blood cell count.

\* Values expressed as mean and (range).

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Table 12 Normal values for biochemical indicators of hepatobiliary disorders in young dogs and cats

Test	Puppies					Kittens		
	1–3 Days	2 Weeks	4 Weeks	8 Weeks	Adult	2 Weeks	4 Weeks	Adult
BSP (%; 30 min)	<5	<5	<5	<5	05	ND	ND	0–3
Bile acids (μmol/L)	<15	<15	<15	<15	0–15	ND	<10	0–10
Bilirubin (mg/dL)	0.5	0.3	0	0.1		0.3	0.2	
ALT (IU/L)	(0.2–1.0) *	(0.1–0.5)	(0–0.1)	(0.1–0.2)	(0–0.04)	(0.1–1.0)	(0.1–0.2)	(0–0.2)
	69	15	21	21		18	17	
AST (IU/L)	(17–337)	(10–21)	(20–22)	(9–24)	(12–94)	(11–24)	(14–26)	(25–91)
	108	20	18	22		18	17	
ALP (IU/L)	(44–194)	(10–40)	(14–23)	(10–32)	(13–56)	(8–48)	(12–24)	(9–42)
	3845	236	144	158		123	111	
GGT (IU/L)	(618–8760)	(176–541)	(135–210)	(144–177)	(4–107)	(68–269)	(90–135)	(10–77)
	1111	24	3	1		1	2	
Protein (g/dL)	(163–3558)	(4–77)	(2–7)	(0–7)	(0–7)	(0–3)	(0–3)	(0–4)
	4.1	3.9	4.1	4.6		4.4	4.8	
Albumin (g/dL)	(3.4–5.2)	(3.6–4.4)	(3.9–4.2)	(3.9–4.8)	(5.4–7.4)	(4.0–5.2)	(4.6–5.2)	(5.8–8.0)
	2.1	1.8	1.8	2.5		2.1	2.3	(2.5–3.0)
Cholesterol (mg/dL)	(1.5–2.8)	(1.7–2.0)	(1.0–2.0)	(2.1–2.7)	(2.1–2.7)	(2.0–2.4)	(2.2–2.4)	
	136	282	328	155		229	361	
Glucose (mg/dL)	(221–204)	(223–344)	(266–352)	(111–258)	(103–299)	(164–443)	(222–434)	(150–270)
	88	129	109	145		117	110	
	(52–127)	(111–146)	(86–115)	(124–272)	(65–110)	(76–129)	(99–112)	(63–144)

From Center SA, Hornbuckle WE, Hoskins JD: The liver and pancreas. In Hoskins JD (ed): Veterinary pediatrics: dogs and cats from birth to six months. Philadelphia, 1990, WB Saunders, [Chapter 8](#).

BSP, Sulfobromophthalein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transferase; ND, not determined.

\* Data expressed as median and (range).

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Table 13 Age-related changes in plasma and urine values in young cats

Laboratory test	4–6 Weeks	7–12 Weeks	13–19 Weeks	20–24 Weeks
Plasma sodium (mEq/L)	152 (147–158)*	151 (144–160)	154 (148–161)	155 (149–162)
Plasma potassium (mEq/L)	4.7 (3.7–5.6)	4.9 (3.6–7.1)	4.7 (3.3–6.5)	4.4 (3.5–6.0)
Plasma chloride (mEq/L)	122 (118–127)	122 (113–128)	123 (118–130)	124 (117–129)
Plasma protein (g/dL)	4.6 (4.2–5.1)	5.2 (4.2–6.7)	5.9 (4.8–6.8)	6.2 (5.4–7.1)
Plasma calcium (mg/dL)	9.7 (8.4–11.0)	9.9 (8.8–11.2)	10.1 (8.8–11.1)	9.9 (8.9–10.9)
Plasma phosphorus (mg/dL)	7.4 (5.0–9.9)	8.2 (6.0–10.5)	7.8 (6.4–9.7)	7.1 (4.9–9.8)
Endogenous creatinine clearance (mL/min/kg)	2.19 (0.1–4.2)	4.1 (2.4–5.7)	3.96 (2.6–5.9)	3.38 (2.1–4.7)
FE sodium (%)	0.25 (0.02–0.46)	0.50 (0.01–1.08)	0.57 (0.34–0.79)	0.55 (0.32–0.75)
FE potassium (%)	12.84 (2.37–25.15)	22.56 (12.61–41.51)	22.64 (11.91–37.64)	23.21 (13.51–31.26)
FE chloride (%)	0.62 (0.25–1.13)	1.10 (0.54–1.79)	1.14 (0.6–1.73)	1.16 (0.76–1.62)
FE calcium (%)	0.39 (0.02–2.11)	0.32 (0.04–2.01)	0.12 (0.02–0.50)	0.06 (0.01–0.13)
FE phosphorus (%)	17.10 (1.51–43.76)	27.43 (9.30–48.88)	30.14 (13.95–53.84)	36.20 (12.35–96.19)
24-Hour urinary protein excretion (mg/dL)	4.62 (0.23–16.43)	9.09 (2.54–27.57)	7.34 (3.15–27.93)	6.80 (2.55–17.28)
Plasma osmolality (mOsm/kg)	307 (275–334)	316 (277–343)	313 (187–333)	309 (264–333)
Urine osmolality (mOsm/kg)	1424 (618–2680)	2432 (1214–3474)	2792 (1408–3814)	2383 (918–3384)
Urine production (mL/kg/24 hr)	25.3 (10.4–66.2)	32.1 (4.3–62.3)	26.2 (12.6–53.4)	20.9 (10.2–30.9)

From Crawford MA: The urinary system. In Hoskins JD (ed): Veterinary pediatrics: dogs and cats from birth to six months. Philadelphia, 1990, WB Saunders, Chapter 10.

FE, Urinary fractional excretion.

\* Values expressed as mean and (range).

Table 14 Erythrograms of foals up to 1 year of age

Age	Packed cell volume (%)	Hemoglobin (g/dL)	Red blood cell count ( $\times 10^6/L$ )	Mean cell volume (fL)	Mean cell hemoglobin concentration (g/dL)
<12 hours	43 $\pm$ 3	15.4 $\pm$ 1.2	10.7 $\pm$ 0.8	40 $\pm$ 2	36 $\pm$ 2
1 day	40 $\pm$ 3	14.2 $\pm$ 1.1	9.9 $\pm$ 0.6	41 $\pm$ 3	35 $\pm$ 2
3 days	38 $\pm$ 3	14.1 $\pm$ 1.3	9.6 $\pm$ 0.7	39 $\pm$ 2	37 $\pm$ 1
1 week	35 $\pm$ 3	13.3 $\pm$ 1.2	8.8 $\pm$ 0.6	39 $\pm$ 2	38 $\pm$ 1
2 weeks	34 $\pm$ 3	12.6 $\pm$ 1.4	8.9 $\pm$ 0.9	38 $\pm$ 2	38 $\pm$ 1
3 weeks	34 $\pm$ 3	12.6 $\pm$ 1.1	9.2 $\pm$ 0.6	37 $\pm$ 2	37 $\pm$ 1
1 month	34 $\pm$ 4	12.5 $\pm$ 1.2	9.3 $\pm$ 0.8	36 $\pm$ 1	37 $\pm$ 1
2 months	37 $\pm$ 4	13.6 $\pm$ 1.5	10.8 $\pm$ 1.7	35 $\pm$ 2	37 $\pm$ 1
3 months	36 $\pm$ 2	13.4 $\pm$ 0.9	10.5 $\pm$ 0.9	35 $\pm$ 1	37 $\pm$ 2
4 months	36 $\pm$ 3	13.4 $\pm$ 1.1	10.4 $\pm$ 0.9	34 $\pm$ 1	38 $\pm$ 2
5 months	35 $\pm$ 3	12.7 $\pm$ 1.2	10.2 $\pm$ 0.6	35 $\pm$ 2	37 $\pm$ 2
6 months	34 $\pm$ 2	12.2 $\pm$ 0.8	9.5 $\pm$ 0.7	36 $\pm$ 2	36 $\pm$ 1
9 months	36 $\pm$ 3	12.6 $\pm$ 1.0	9.4 $\pm$ 0.8	39 $\pm$ 2	35 $\pm$ 1
12 months	36 $\pm$ 3	13.3 $\pm$ 1.0	9.5 $\pm$ 0.7	38 $\pm$ 2	37 $\pm$ 2

From Harvey JW, Asquith RL, McNulty PK, et al: Haematology of foals up to one year old. Equine Vet J 1984; 16:347–353.

Values expressed as mean  $\pm$  1 SD.

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Table 15 Hemostasis values for healthy horses

Laboratory test	Mean ± SD	Range
Prothrombin time (seconds)	9.8 ± 0.34	9.2–10.5
APTT (seconds)	46.5 ± 9.2	31–93
Antithrombin III (%)	193 ± 28	115–239
Plasminogen (%)	110 ± 23	63–146
Fibrinogen (mg/dL)	192 ± 80	120–490
Fibrin(ogen) degradation products (µg/dL)	24 ± 19	0–64
Platelet count ( $\times 10^5/\mu\text{L}$ )	1.33 ± 0.34	0.89–2.32

From Prasse KW, Allen D Jr, Moore JN, et al: Evaluation of coagulation and fibrinolysis during the prodromal stages of carbohydrate-induced acute laminitis in horses. *Am J Vet Res* 1990;51:1950–1955.  
 APTT, Activated partial thromboplastin time.

Table 16 Leukograms of foals up to 1 year of age

Age	Total WBC ( $\times 10^3/\mu\text{L}$ )	Neutrophils ( $\times 10^3/\mu\text{L}$ )	Lymphocytes ( $\times 10^3/\mu\text{L}$ )	Monocytes ( $\times 10^3/\mu\text{L}$ )	Eosinophils ( $\times 10^3/\mu\text{L}$ )	Basophils ( $\times 10^3/\mu\text{L}$ )
<12 hours	9.5 ± 2.44	7.94 ± 2.22	1.34 ± 0.60	0.19 ± 0.12	0	0.002 ± 0.007
1 day	8.44 ± 1.77	6.80 ± 1.72	1.43 ± 0.42	0.19 ± 0.10	0.11 ± 0.027	0.003 ± 0.010
3 days	7.55 ± 1.50	5.70 ± 1.44	1.45 ± 0.36	0.32 ± 0.13	0.045 ± 0.062	0.032 ± 0.046
1 week	9.86 ± 1.79	7.45 ± 1.55	2.10 ± 0.63	0.27 ± 0.11	0.028 ± 0.042	0.058 ± 0.069
2 weeks	8.53 ± 1.68	6.00 ± 1.54	2.22 ± 0.45	0.24 ± 0.13	0.063 ± 0.063	0.012 ± 0.021
3 weeks	8.57 ± 1.90	5.66 ± 1.64	2.59 ± 0.63	0.22 ± 0.10	0.078 ± 0.066	0.026 ± 0.032
1 month	8.14 ± 2.02	5.27 ± 2.00	2.46 ± 0.45	0.29 ± 0.17	0.121 ± 0.148	0.016 ± 0.032
2 months	9.65 ± 2.13	5.70 ± 1.88	3.46 ± 0.63	0.31 ± 0.15	0.092 ± 0.092	0.018 ± 0.039
3 months	11.69 ± 2.51	6.43 ± 1.96	4.73 ± 1.21	0.38 ± 0.19	0.184 ± 0.181	0.018 ± 0.028
4 months	10.18 ± 1.99	4.78 ± 1.36	4.70 ± 1.31	0.32 ± 0.17	0.353 ± 0.319	0.018 ± 0.027
5 months	10.07 ± 2.29	4.60 ± 1.90	4.92 ± 1.48	0.27 ± 0.12	0.272 ± 0.152	0.010 ± 0.027
6 months	9.03 ± 1.13	4.00 ± 0.84	4.53 ± 0.74	0.23 ± 0.11	0.247 ± 0.150	0.014 ± 0.024
9 months	8.68 ± 1.19	3.82 ± 0.78	4.39 ± 1.10	0.22 ± 0.10	0.234 ± 0.232	0.021 ± 0.024
12 months	9.19 ± 1.36	4.28 ± 0.81	4.27 ± 1.13	0.20 ± 0.12	0.339 ± 0.221	0.019 ± 0.037

From Harvey JW, Asquith RL, McNulty PK, et al: Haematology of foals up to one year old. *Equine Vet J* 1984;16:347–353.  
 Values are expressed as mean ± 1 SD.

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Table 17 Percent fractional urinary excretion of electrolytes in cows

Electrolyte	Summer	Autumn	Winter	Spring
Sodium	1.3 (0.13)*	0.68 (0.09)	0.63 (0.13)	0.33 (0.06)
Potassium	55.16 (4.12)	47.41 (3.80)	32.44 (3.15)	21.65 (3.41)
Chloride	2.12 (0.11)	2.25 (0.12)	1.80 (0.17)	0.68 (0.12)
Osmolality	4.70 (0.25)	3.84 (0.19)	3.71 (0.16)	1.82 (0.18)

From Itoh N: Fractional electrolyte excretion in adult cows: establishment of reference ranges and evaluation of seasonal variations. *Vet Clin Pathol* 1989;18:86–87.

\* Data expressed as mean and (standard error).

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Table 18 Normal percent fractional excretion of electrolytes in the urine of domestic animals<sup>\*</sup>

Electrolyte	Dog	Cat	Horse	Cow	Sheep
Sodium	0–0.7	0.24–1.0	0.02–1.0	0.2–1.43	0–0.071
Potassium <sup>†</sup>	0–20	6.7–23.9	15–65	15–63	80–180
Chloride	0–0.8	0.41–1.3	0.04–1.6	0.4–2.3	0–4.7
Phosphorus	3–39	17–73		0–0.2	0–0.53

<sup>†</sup> The Fc for potassium in herbivorous animals is largely dependent on the diet.

<sup>\*</sup> Calculated using the formula:  $\% \text{CrClFE}(\%) = \frac{\text{Cr serum}}{\text{Cr urine}} \times \frac{\text{E urine}}{\text{E serum}} \times 100$  Cr, Creatinine; E, electrolyte.